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PROVISIONAL SPECIFICATION

for the invention entitled:

"Molecular mimics and methods for their production"

The invention is described in the following statement:

MOLECULAR MIMICS AND METHODS FOR THEIR PRODUCTION

FIELD OF THE INVENTION

[0001] THIS INVENTION relates generally to short chain peptides that have been constrained to adopt an alpha helical conformation and to their use as alpha helical scaffolds for directing amino acid side chains into positions analogous to those found in longer chain alpha helical peptides and for attaching peptidic or non-peptidic appendages in order to mimic side chains of longer alpha helical peptides. More particularly the invention relates to alpha helical cyclic pentapeptides and their use as alpha helical scaffolds or macrocyclic alpha helical modules, either alone, or within longer chain peptides or attached to other macrocyclic peptides or attached to non-peptidic structures, for the purpose of mimicking naturally occurring peptides or proteins, and as agonists or antagonists of the biological activity of naturally-occurring peptides or proteins or for the preparation of new materials.

[0002] Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

BACKGROUND OF THE INVENTION

[0003] The alpha helix is a fundamental structural unit in the fabric of proteins, with 30% of all amino acids in proteins occurring in α-helices.[1] When helical sequences of amino acids are exposed on an exterior surface of a protein, the helix frequently interacts with another protein, a segment of DNA or of RNA.[2,3] This biomolecular recognition is central to a large range of biological processes, for example those summarized in Table 1. In most cases however only a few α-helical turns are actually involved in the molecular recognition. For example, transcriptional regulators (e.g. p53, NF-kBp65, VP16c)[4-6] apoptosis regulators (e.g. Bak)[7] and RNA-transporter proteins (e.g. Rev)[8] all contain a short α-helical sequence of only 2-4 turns that mediates function by direct interaction with a receptor.

TABLE 1

SOME BIOLOGICAL PROCESSES MEDIATED BY INTERACTION OF ALPHA-HELICES WITH OTHER BIOMOLECULES

α-Helical Peptide	Biological target	Process Mediated	Reference
Protein-DNA interactions			
Zif268	G/C rich major groove	DNA transcription	9
Protein-RNA interactions			
HIV Reverse Transcriptase	Rev Response Element (RRE)	RNA reverse transcription	10
λ–N peptide	BoxB RNA	Transcriptional anti-termination	10
P22 peptides	BoxB RNA	Transcriptional anti-termination	10
Protein-Protein interactions			
p53	HDM2	Tumor Suppressor silencing	4
Bak	Bcl-X_L	Apoptosis Regulation	7
VHL peptide	Elongin C	DNA transcription	11
VP16 activation domain	$HTAF_{II}31$	DNA transcription	12
hPTH	hPTHrP	Calcium homeostasis	13
Dynorphin A	κ,δ-Opioid receptors	Pain signal transmission	14,15
Apolipoprotein-E	LDL receptor	Lipid metabolism, cholesterol homeostasis	16
Neuropeptide-Y	NPY receptors	Multiple functions	17
Galanin	Gal receptors	Multiple functions	18
Corticotropin Releasing Factor	CRF receptors	Stress responses	19
Calcitonin Gene Related Peptide	CGRP receptors	Multiple functions	20
Nociceptin	ORL1 receptor	Pain transmission	
Vasointestinal Peptide	$VPAC_{1\ \&\ 2}$	Multiple functions	21
Nuclear Coactivators (eg. SRC1, GRIP1)	Nuclear Receptors	DNA Transcription	22,23

[0004] Short peptide sequences of less than 15 amino acids that correspond to these helical protein regions are not thermodynamically stable structures in water when removed from their protein environments.[24,25].

[0005] Attempts to stabilise short α-helical peptides have met with limited success to date. Examples of methods used to stabilize alpha helicity in peptides longer than 15 residues are helix-nucleating templates[26-29], metals[30-35], unnatural amino acids[36,37], non-covalent side chain constraints[38,39] and covalent side chain linkers (e.g. disulfide- [40,41], hydrazone-[42], lactam- [43-50], aliphatic linkers[51-53]). Although mimics of short alpha helical segments have remained elusive, some recent attempts have been reported using non-peptidic oligoamide and terphenyl scaffolds that project 2-3 substituents into similar three dimensional space as the side chains of an α-helix [54-56].

[0006] Helix nucleating templates are organic molecules at the N- or C-terminus of a peptide which can make hydrogen bonds with the first or last four NH or C=O groups in the peptide, and thus nucleate helicity throughout the rest of the peptide. Such a task is not trivial due to the specific position, pitch and orientation of the required NH or C=O groups. Several attempts have had some success, these include Kemp's triacid, cyclic proline molecules, [26, 57-61], Mueller's Cage compound[62], Bartlett's cap[28], and Kahn's cap[63]. There have also been some attempts to synthesise capping groups by replacing a hydrogen bond with a covalent link as in the case of Satterthwait's cap[64].

[0007] Transition metals[30-35] are often found in proteins serving both catalytic and structural roles. By exploiting the ability of transition metals such as Cu^{2+}, Zn^{2+}, Cd^{2+}, Ru^{3}, Pd^{2+} to bind both acidic and basic residues it has been possible to achieve helix stabilisation. Chelation of metals to donor groups generally yields ~1 kcal/mol^{-1} in helix stabilisation; however stabilisation is very dependent on solvent, salt concentration and pH.

[0008] Unnatural amino acids have also been reported to favour helix stabilisation. In general n-alkyl substitution, α,α- and γγ-disubstitution increases helix stability. β,β-Disubstitution reduces helicity, and β–tertiary substitution totally abolishes helix propensity, thus it appears the helix is quite sensitive to steric effects at the β-position[65]. α-Aminoisobutyric acid (Aib) in particular is known to stabilise α- and 3_{10}-helical conformations and has been used to improve the biological activity of several peptides. Nociceptin analogues containing 1 or 2 Aib residues resulted in 10-15 fold increases in potency and affinity (K_i= 0.02nM)[66]. Similarly an analogue of p53 containing Aib and 1-aminocyclopropanecarboxylic acid (Ac_3c) yielded a peptide 1735 more active than the native peptide[67]. Finally when Aib was substituted into deltorphin-C analogues a 10-fold K_i increase in selectivity was obtained for δ vs μ opioid receptor subtypes[68].

[0009] Disulfide bridges have been employed to stabilise helices *via* two methods. The first involves the use of a modified, unnatural amino acid D,L 2-amino-6-mercaptohexanoic acid placed at the i^{th} (D) and $i+7^{th}$ (L) residues to stabilise two turns of an α-helix[41]. The second approach involves using a D-cysteine (*i*) and L-cysteine (*i*+3) disulfide to stabilise a single α-helical turn. This approach was successful to a certain extent, however the conformation was quite solvent dependent[40]. It has recently been reported that this approach was used to constrain the SRC-1 peptide, which is known to adopt an α-helical conformation in the estrogen receptor-α, and inhibit this receptor with a K_i of 25nM [69].

[0010] Lactam bridges have often been used to increase helicity and turn conformations in long peptides. They generally involve the covalent amide linkage of the side chains of lysine/ornithine residues with the side chains of aspartic/glutamic acid residues at either *i* to *i*+3 or *i* to *i*+4 positions. These constraints although initially examined in model peptides have been applied to numerous biological targets in which the bioactive conformation is deemed to be helical. In general this constraint has been employed in relatively long sequences (15-30 residues) generally to create monocyclic analogues, but in some cases, up to three lactam bridges have been included. Some examples of their use include PTH, NPY, CRF, GCN4, Galanin and Dynorphin-A. Despite their inception over 10 years ago, there is still a lack of consensus over which residue combinations are the best, although it appears *i* to *i*+4 spacing is optimal for α-helicity. Early pioneering work by Taylor[48] suggested Lys→Asp was the optimal combination, however, later work by Houston identified Glu→Lys as optimal, although this study totally neglected to use aspartic acid[70]. More recent work by Taylor has involved using overlapping lactam bridges to yield a highly rigid hexapeptide α-helix, highly resistant to chemical and thermal degradation[45], and with some templating capability[71]. However, this hexapeptide scaffold is limited for general application as a template since only two of six residues are available for interaction with a biological target. The synthesis and properties of side-chain lactam bridged peptides, their alpha helical nature, functional activity and potential for improved proteolysis resistance has recently been reviewed[43].

[0011] Modified lactam-type bridges are generally spaced *i* to *i*+7 therefore requiring longer linkers, thus aspartic/glutamic acid, and/or diaminopropionic acid residues provide a convenient functionality to which linkers can be attached. Some of these have included diaminopentane linkers joined to two glutamic acids[53], 4-(aminomethyl)-phenylacetic acid

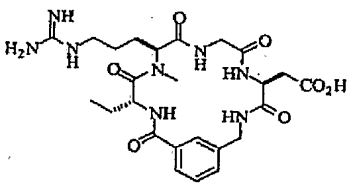
linked via aspartic acid and 1,3-diaminopropionic acid[49], or alternately 4-(aminomethyl)-phenylazobenzoic acid joined to the N- and C-terminus of an octapeptide. The two former methods resulted in reasonably stable helices, whilst the latter resulted in a 3_{10} helical/random coil conformation depending on the cis/trans isomerization of the azo linkage.

[0012] Ring closing metathesis has been used in helix stabilisation. Pioneered by Grubbs[72], this approach has been utilised with allyl-modified serine/homoserine residues in $i \rightarrow i+4$ fashion. It has not been overly successful in stabilising α-helicity, although some 3_{10} stabilisation was observed. Other approaches have incorporated both S- and R-α-methyl-α-allylglycine, along with the α-homoallyl and α-homohomoallyl derivatives, positioned at either $i \rightarrow i+4$ or $i \rightarrow i+7$ [51]. It was found that the R-isomer at the *i* position and the S-isomer at the $i+7$ position, with an 11 carbon link provided 44% helix stability compared to the uncyclised peptide.

[0013] Non-peptidic mimicry of α-helices has been rare, with only a few examples reported. The first reported non-peptidic helix mimetics were 1,1,6-trisubstituted indanes, that when coupled to an amino acid were capable of presenting three side chains in a helical like conformation. When applied as tachykinin mimetics, they had micromolar affinity for NK and NK_3 receptors[73]. These type of molecules were recently applied to magainin mimicry, and whilst they were capable of killing bacterial strains they still maintained high haemolytic activity[74]. Recently Kahne and co-workers developed a pentasaccharide helix mimetic based on GCN4 which bound DNA with micromolar affinity[75]. By far the most successful approach to non-peptidic α-helix mimicry has been achieved by Hamilton and co-workers who have successfully developed two generic types of molecules – terphenyls and oligoamides capable of mimicking the *i*, $i+4$, $i+7$ side chains on one face of an α-helix. These mimetics have been successfully applied to inhibition of HIV gp41 mediated viral fusion with an IC_{50} of 15.7μg/ml[76], and also inhibit Bak/Bcl-X_L complex with low micromolar to nanomolar efficiency[77,78].

[0014] There have been no previous reports of cyclic pentapeptides adopting alpha helices on their own. Usually cyclic pentapeptides have been found to mimic the smaller beta or gamma turns of peptides and proteins. There are numerous examples of cyclic peptides that mimic beta or gamma turns reported in the literature as demonstrated by several reviews[73-81]. A prime example is synthetic compound 1 which is a cyclic pentapeptide containing the RGD tripeptide sequence. This compound is a potent

glycoprotein IIb/IIIa antagonist and orally bioavailable antithrombotic and antitumor agent[73, 82, 83]. Compound 1 provides a demonstration of how the simple insertion into a cyclopeptide of a rigid amino acid as a conformational constraint can result in favourable biological and pharmacological properties; and a number of its derivatives are in advanced clinical trials. For example, in phase III clinical trials, the cyclic RGD-containing heptapeptide drug eptifibatide (Integrilin) has been shown to reduce the incidence of cardiac events in patients at risk of abrupt vessel closure after coronary angioplasty[84].



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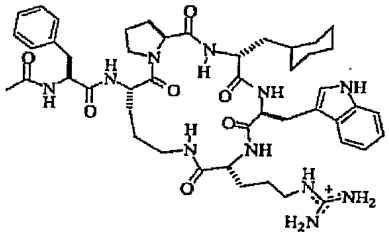

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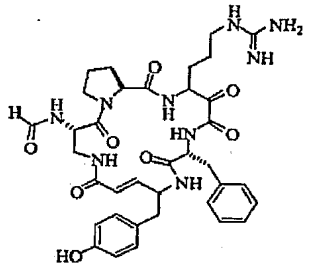


3

[0015] Constraints do not need to be complex, as shown in compound 2 where an ornithine (or lysine) side chain is used to form the macrocycle. This constraint, in conjunction with proline and D-cyclohexylalanine constraints, induces intramolecular hydrogen bonding that confers potent antagonism (IC_{50} 10 nM) against human C5a receptors on polymorphonuclear leukocytes both *in vitro* and *in vivo*[85]. C5a antagonists are expected to be useful for combating inflammatory diseases.

[0016] Cyclotheonamide A (Compound 3) is a 19-membered cyclic pentapeptide possessing α-keto amide and *trans*-4-aminobutenoyl constraints. It was isolated from the marine sponge *Theonella* sp. and was shown to inhibit the serine proteases thrombin (Ki 180 nM) and trypsin (Ki 23 nM). The NMR solution structure of compound 3 was recently found to be the same in water as those found in the solid state when bound to trypsin and thrombin[86], suggesting that this natural product is pre-organized for enzyme binding, and that selectivity is associated with the positioning of the D-Phe side chain.

[0017] Lactam bridges ($i \rightarrow i+3$, $i \rightarrow i+4$, $i \rightarrow i+7$) have previously been reported to increase alpha helicity in longer peptides, although the literature is very inconsistent about their capacity to do so[43-51]. There have been no reports of cyclic pentapeptides adopting alpha helical structures.

[0018] The synthesis and conformation of multicyclic alpha helical peptides comprising three repeats of a heptapeptide constrained by a side-chain to side-chain lactam bridge in $(i)\rightarrow(i+4)$ positions has been reported[48,114]. These studies showed that spaced cyclic moieties in a peptide can induce or stabilize alpha helicity.

[0019] Conformational restrictions in the form of $(i)\rightarrow(i+4)$ lactam bridges incorporated into known peptide sequences to induce helical conformation have also been reported[115]. Three constrained helical 31-residue peptides derived from human parathyroid hormone and containing 1, 2 or 3 cyclic moieties were shown to be potent agonists of the parathyroid hormone and parathyroid hormone-related protein receptor

[0020] There are many commercially important peptides that are known to adopt alpha helical structures that would benefit from improved structural stabilisation and improved resistance to proteolysis. Some examples include calcitonin which has been launched for the treatment of osteoporosis, the parathyroid hormone which is in phase II clinical trials for the treatment of osteoporosis, a substance-P/saporin conjugate which is in preclinical trials for the treatment of pain and conantokin-G which is under development for the treatment of epilepsy (Pharmaprojects, 2004).

[0021] There is a need for stabilised short peptide alpha helices that can mimic biological molecules or that can be incorporated into non-peptidic compounds to mimic biological molecules. Such peptides could potentially be valuable as chemical and biological probes, pharmaceuticals, biotechnology products such as vaccines, or diagnostic agents, new components of biopolymers and industrial agents.

SUMMARY OF THE INVENTION

[0022] This invention is predicated in part on the unexpected discovery that certain short chain peptides, which comprise at least one macrocyclic pentapeptide unit, are highly alpha helical in their own right in water even when subjected to denaturing conditions. This discovery has been reduced to practice in novel alpha helical compounds, non peptidic structures containing them and in methods for their preparation and use, as described hereinafter.

DETAILED DESCRIPTION OF THE INVENTION

[0023] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group

of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0024] Advantageously, at least one embodiment of the present invention provides compounds comprising at least one macrocyclic moiety, particularly a cyclic pentapeptide moiety, which has surprising alpha helicity in water, even under strong protein denaturing conditions such as high temperature (e.g., 40-80°C), or the presence of up to 8M guanidine hydrochloride, or the presence of proteolytic enzymes such as trypsin.

[0025] According to one aspect of the present invention there is provided a compound comprising at least one alpha helical cyclic peptide, wherein the peptide consists essentially of a sequence of five amino acid residues having a first terminal residue and a second terminal residue that are separated by an intervening sequence of three amino acid residues, and wherein the side chains of the first and second terminal residues are linked to each other, with the proviso that when the compound comprises a single cyclic peptide it is selected from a compound that consists essentially of the single peptide or a compound that comprises the single peptide and a non-peptide moiety and that when the compound comprises two or more cyclic peptides, at least two of these are located immediately adjacent to each other.

[0026] As used herein "alpha helical" refers to a three dimensional structural conformation which is analogous to those found in proteins and polypeptides. The alpha helix conformation found in naturally occurring proteins and polypeptides has its side chains extending to the outside of the structure, has a complete turn every 3.6 amino acids, is right-handed and typically has hydrogen bonding between the carbonyl groups of the amide bond and an amide N-H group 4 amino acids further on in the sequence. The cyclic peptides of the present invention have a helicity calculated from molar ellipticities obtained from circular dichroism spectroscopy (CD spectroscopy) and are expressed as a percentage of the theoretical helicity obtainable for that peptide.

[0027] As used herein, the term "amino acid" refers to compounds having an amino group and a carboxylic acid group. An amino acid may be a naturally occurring amino acid or non-naturally occurring amino acid and may be a proteogenic amino acid or a non-proteogenic amino acid. The amino acids incorporated into the amino acid sequences of the present invention may be L-α-amino acids, D-α-amino acids or mixtures thereof.

[0028] In some embodiments, the cyclic peptides of the invention are linked directly or indirectly to non-peptide moieties. Such moieties include, but are not limited to,

aldehydes, toxins; drugs; polysaccharides; nucleotides; oligonucleotides; labels such as radioactive substances (e.g. ^{111}In, ^{125}I, . ^{131}I, ^{99m}Tc, ^{212}B, ^{90}Y, ^{186}Rh); biotin; fluorescent tags; imaging reagents (e.g. those described in U.S. Pat. No. 4,741,900 and U.S. Pat. No. 5,326,856); hydrocarbon linkers (e.g., an alkyl group or derivative thereof) conjugated to a moiety providing for attachment to a solid substratum, or to a moiety providing for easy separation (e.g., a hapten recognized by an antibody bound to a magnetic bead), etc. Linkage of the peptide to the non-peptide moiety may be by any of several well-known methods in the art.

[0029] Suitable naturally occurring proteogenic amino acids are shown in Table 2 together with their one letter and three letter codes.

TABLE 2

Amino Acid	one letter code	three letter code
L-alanine	A	Ala
L-arginine	R	Arg
L-asparagine	N	Asn
L-aspartic acid	D	Asp
L-cysteine	C	Cys
L-glutamine	Q	Gln
L-glutamic acid	E	Glu
glycine	G	Gly
L-histidine	H	His
L-isoleucine.	I	Ile
L-leucine	L	Leu
L-lysine	K	Lys
L-methionine	M	Met
L-phenylalanine	F	Phe
L-proline	P	Pro
L-serine	S	Ser
L-threonine	T	Thr
L-tryptophan	W	Trp
L-tyrosine	Y	Tyr
L-valine	V	Val

[0030] Suitable non-proteogenic or non-naturally occurring amino acids may be prepared by side chain modification or by total synthesis. Examples of side chain modifications contemplated by the present invention include, but are not limited to modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2,4,6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

[0031] The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

[0032] The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

[0033] Sulfydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulfides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulfonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

[0034] Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulfenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

[0035] Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carboethoxylation with diethylpyrocarbonate.

[0036] Examples of incorporating unnatural amino acids and derivatives during protein synthesis include, but are not limited to, use of norleucine, t-butylglycine,

norvaline, phenylglycine, ornithine, sarcosine, 2-thienyl alanine and/or D-isomers of amino acids. Examples of suitable non-proteogenic or non-naturally occurring amino acids contemplated herein is shown in Table 3.

TABLE 2

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
L-N-methylaspartic acid	Nmasp	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
cyclohexylalanine	Chexa	L-N-methylglutamic acid	Nmglu
cyclopentylalanine	Cpen	L-N-methylhistidine	Nmhis
D-alanine	Dal	L-N-methylisolleucine	Nmile
D-arginine	Darg	L-N-methylleucine	Nmleu
D-aspartic acid	Dasp	L-N-methyllysine	Nmlys
D-cysteine	Dcys	L-N-methylmethionine	Nmmet
D-glutamine	Dgln	L-N-methylnorleucine	Nmnle
D-glutamic acid	Dglu	L-N-methylnorvaline	Nmnva
D-histidine	Dhis	L-N-methylornithine	Nmorn
D-isoleucine	Dile	L-N-methylphenylalanine	Nmphe
D-leucine	Dleu	L-N-methylproline	Nmpro
D-lysine	Dlys	L-N-methylserine	Nmser
D-methionine	Dmet	L-N-methylthreonine	Nmthr
D-ornithine	Dorn	L-N-methyltryptophan	Nmtrp
D-phenylalanine	Dphe	L-N-methyltyrosine	Nmtyr
D-proline	Dpro	L-N-methylvaline	Nmval
D-serine	Dser	L-N-methylethylglycine	Nmetg

Non-conventional amino acid	Code	Non-conventional amino acid	Code
D-threonine	Dthr	L-N-methyl-t-butylglycine	Nmtbug
D-tryptophan	Dtrp	L-norleucine	Nle
D-tyrosine	Dtyr	L-norvaline	Nva
D-valine	Dval	α-methyl-aminoisobutyrate	Maib
D-α-methylalanine	Dmala	α-methyl- -aminobutyrate	Mgabu
D-α-methylarginine	Dmarg	α-methylcyclohexylalanine	Mchexa
D-α-methylasparagine	Dmasn	α-methylcylcopentylalanine	Mcpen
D-α-methylaspartate	Dmasp	α-methyl-α-napthylalanine	Manap
D-α-methylcysteine	Dmcys	α-methylpenicillamine	Mpen
D-α-methylglutamine	Dmgln	N-(4-aminobutyl)glycine	Nglu
D-α-methylhistidine	Dmhis	N-(2-aminoethyl)glycine	Naeg
D-α-methylisoleucine	Dmile	N-(3-aminopropyl)glycine	Norn
D-α-methylleucine	Dmleu	N-amino-α-methylbutyrate	Nmaabu
D-α-methyllysine	Dmlys	α-napthylalanine	Anap
D-α-methylmethionine	Dmmet	N-benzylglycine	Nphe
D-α-methylornithine	Dmorn	N-(2-carbamylethyl)glycine	Ngln
D-α-methylphenylalanine	Dmphe	N-(carbamylmethyl)glycine	Nasn
D-α-methylproline	Dmpro	N-(2-carboxyethyl)glycine	Nglu
D-α-methylserine	Dmser	N-(carboxymethyl)glycine	Nasp
D-α-methylthreonine	Dmthr	N-cyclobutylglycine	Ncbut
D-α-methyltryptophan	Dmtrp	N-cycloheptylglycine	Nchep
D-α-methyltyrosine	Dmty	N-cyclohexylglycine	Nchex
D-α-methylvaline	Dmval	N-cyclodecylglycine	Ncdec
D-N-methylalanine	Dnmala	N-cylcododecylglycine	Ncdod
D-N-methylarginine	Dnmarg	N-cyclooctylglycine	Ncoct
D-N-methylasparagine	Dnmasn	N-cyclopropylglycine	Ncpro

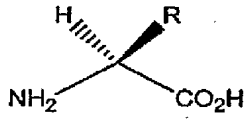
Non-conventional amino acid	Code	Non-conventional amino acid	Code
D-N-methylaspartate	Dnmasp	N-cycloundecylglycine	Ncund
D-N-methylcysteine	Dnmcys	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylglutamine	Dnmgln	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamate	Dnmglu	N-(3-guanidinopropyl)glycine	Narg
D-N-methylhistidine	Dnmhis	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylisoleucine	Dnmile	N-(hydroxyethyl))glycine	Nser
D-N-methylleucine	Dnmleu	N-(imidazolylethyl))glycine	Nhis
D-N-methyllysine	Dnmlys	N-(3-indolylyethyl)glycine	Nhtrp
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyl-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
L-*t*-butylglycine	Tbug	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-ethylglycine	Etg	N-(thiomethyl)glycine	Ncys
L-homophenylalanine	Hphe	penicillamine	Pen
L-α-methylarginine	Marg	L-α-methylalanine	Mala
L-α-methylaspartate	Masp	L-α-methylasparagine	Masn
L-α-methylcysteine	Mcys	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylglutamine	Mgln	L-methylethylglycine	Metg
L-α-methylhistidine	Mhis	L-α-methylglutamate	Mglu
L-α-methylisoleucine	Mile	L-α-methylhomophenylalanine	Mhphe

Non-conventional amino acid	Code	Non-conventional amino acid	Code
L-α-methylleucine	Mleu	N-(2-methylthioethyl)glycine	Nmet
L-α-methylmethionine	Mmet	L-α-methyllysine	Mlys
L-α-methylnorvaline	Mnva	L-α-methylnorleucine	Mnle
L-α-methylphenylalanine	Mphe	L-α-methylornithine	Morn
L-α-methylserine	Mser	L-α-methylproline	Mpro
L-α-methyltryptophan	Mtrp	L-α-methylthreonine	Mthr
L-α-methylvaline	Mval	L-α-methyltyrosine	Mtyr
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	L-N-methylhomophenylalanine	Nmhphe
1-carboxy-1-(2,2-diphenyl ethylamino)cyclopropane	Nmbc	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe

[0037] As used herein, "amino acid side chain" or "side chain" refers to the characterising substituent of the amino acid. This term refers to the substituent bound to the α-carbon of either a natural or non-natural α-amino acid. For example, the characterising substituents of some naturally occurring amino acids are shown in Table 4.

TABLE 4

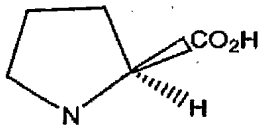
THE PROTEINOGENIC AMINO ACIDS



Amino acid	-R
Alanine	$-CH_3$
Arginine	$-(CH_2)_3NHC(=NH)NH_2$
Asparagine	$-CH_2CONH_2$
Aspartic acid	$-CH_2CO_2H$
Cysteine	$-CH_2SH$

Amino acid	-R
Glutamine	$-(CH_2)_2CONH_2$
Glutamic acid	$-(CH_2)_2CO_2H$
Glycine	-H
Histidine	$-CH_2$(4-imidazolyl)
Isoleucine	$-CH(CH_3)CH_2CH_3$
Leucine	$-CH_2CH(CH_3)_2$
Lysine	$-(CH_2)_4NH_2$
Methionine	$-(CH_2)_2SCH_3$
Phenylalanine	$-CH_2Ph$
Serine	$-CH_2OH$
Threonine	$-CH(CH_3)OH$
Tryptophan	$-CH_2$(3-indolyl)
Tyrosine	$-CH_2$(4-hydroxyphenyl)
Valine	$-CH(CH_3)_2$

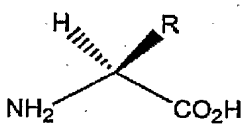
[0038] Another naturally occurring amino acid is proline.



in which the α-side chain terminates in a bond with the amino acid amine nitrogen atom. Some non-limiting examples of characterising substituents of non-naturally occurring amino acids are shown in Table 5:

TABLE 5

NON-NATURAL AMINO ACIDS



Amino acid	-R
α-aminobutyric acid	$-CH_2CH_3$
ornithine	$-(CH_2)_3NH_2$
cyclohexylalanine	$-CH_2C_6H_{10}$
cyclopentylalanine	$-CH_2C_5H_8$
norvaline	$-CH_2CH_2CH_3$
norleucine	$-(CH_2)_3CH_3$

[0039] Preferably the cyclic peptide is a macrocycle formed by consecutively linking at least 18 to 22 atoms, wherein the first and last atoms are bonded to one another to form a ring. In a preferred embodiment the macrocycle is formed from 19 to 21 atoms, especially preferred are macrocycles formed from 20 atoms. In some embodiments, the first and fifth amino acid positions are occupied by alpha amino acids. In these embodiments, the resulting macrocycle ring size is preferably 18-22 atoms, more preferably 20 atoms. In particular, where the first and fifth amino acid positions are occupied by Lys and Asp, respectively, the resulting macrocycle ring size is preferably 18-22 atoms, more preferably 20 atoms. It will be apparent to persons skilled in the art that modifications to the substituents at the first and fifth positions will result in a slightly different optimal macrocycle requirements.

[0040] The two amino acid side chains of the first and second terminal residues defined above may be linked in any suitable manner to form a cyclic pentapeptide. Preferably the side chains are linked by a covalent bond either directly or through a linker. In a preferred embodiment the side chains are covalently linked to one another without an intervening linker, for example, by formation of a lactam bridge between a side chain carboxylic acid group and a side chain amino group or a disulfide bond between two side chain thiol groups. In an especially preferred embodiment, a carboxylic acid in the side

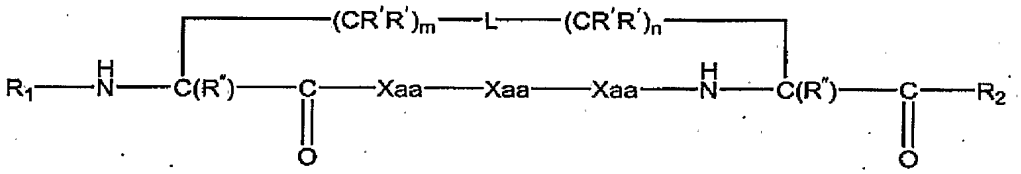
chain of one amino acid residue is reacted with an amine in the side chain of a second amino acid residue to form an amide bond or lactam bridge.

[0041] Preferably one of the amino acid residues having a side chain participating in the linkage is selected from L-aspartic acid, L-glutamic acid, D-aspartic acid, D-glutamic acid, L-α-methyl-aspartic acid, L-α-methylglutamic acid, D-α-methylaspartic acid and D-α-methyl-glutamic acid, and the other amino acid residue having a side chain participating in the linkage is selected from L-lysine, L-ornithine, D-lysine, D-ornithine, L-α-methyllysine, D-α-methyllysine, L-α-methylornithine and D-α-methylornithine. Preferably the amide bond is formed by reaction of an L-aspartic acid or L-glutamic acid with an L-lysine or L-ornithine.

[0042] In a preferred embodiment of the invention the amino acid residues in the sequence are D- or L-α-amino acids, especially L-α-amino acids.

[0043] In another aspect of the invention there is provided a compound having the following formula (I):

(I)



wherein each Xaa is independently selected from any amino acid residue;

R_1 is selected from H, an N-terminal capping group, a non-peptidic group or a group that mimics an amino acid side chain;

R_2 is selected from H, a C-terminal capping group, a group that mimics an amino acid side chain or a group that activates the terminal carboxylic acid carbonyl group to nucleophilic substitution;

each R′ and R″ are independently selected from H, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10}cylcoalkyl, C_5-C_{10}cycloalkenyl, -OH, -OC_1-C_{10} alkyl, -NH_2, -NH(C_1-C_{10}alkyl), -N(C_1-C_{10}alkyl)$_2$, C_6-C_{12}aryl, C_3-C_{10}heterocyclyl, C_5-C_{10}heteroaryl and halo;

L is selected from –NH-C(O)-, –C(O)-NH-, -S-S-, -CH(OH)CH_2-, CH_2CH(OH)-,

-CH=CH-, -CH_2-CH_2-, -NH-CH_2- -CH_2-NH-, -CH_2-S-, -S-CH_2-, -C(O)-CH_2-, -CH_2-C(O)-, -$S(O)_t$-NH-, -NH-$S(O)_t$-, CH_2-P(=O)(OH)- and –P(=O)(OH)-CH_2-;

m is an integer from 1 to 4 and

n is an integer from 1 to 4

wherein m + n = 4, 5 or 6 and wherein when m is 2, n is not 3 and when m is 3, n is not 2.

[0044] As used herein, the term "alkyl" refers to a saturated, straight or branched chain hydrocarbon group, preferably having 1 to 10 carbon atoms. Examples of suitable alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, butyl, sec-butyl, tert-butyl, pentyl, 2-methylbutyl, 3-methylbutyl, 4-methylbutyl, hexyl, 2-ethylbutyl, heptyl, octyl, nonyl and decyl. Preferred alkyl groups have 1 to 6 carbon atoms. Especially preferred alkyl groups have 1 to 3 carbon atoms.

[0045] As used herein, the term "alkenyl" refers to a straight or branched chain hydrocarbons containing at least one carbon-carbon double bond. Suitable alkenyl groups having 2 to10 carbon atoms and include, but are not limited to, vinyl, allyl, 1-methylvinyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl and decenyl. Preferred alkenyl groups have 2 to 6 carbon atoms. Especially preferred alkenyl groups have 2 or 3 carbon atoms.

[0046] As used herein, the term "alkynyl" refers to straight chain hydrocarbons containing at least one carbon-carbon triple bond. Suitable alkynyl groups having 2 to 10 carbon atoms include, but not limited to, ethylnyl, 1-propynyl, 2-propynyl, butynyl, pentynyl, hexynyl, heptynyl, octynyl, nonynyl and decynyl. Preferred alkynyl groups have 2 to 6 carbon atoms. Especially preferred alkynyl groups have 2 or 3 carbon atoms.

[0047] As used herein, "halo" is intended to include fluoro, chloro, bromo and iodo.

[0048] As used herein, the term "cycloalkyl" refers to saturated mono- or poly- cyclic hydrocarbon groups. Suitable cycloalkyl groups having 3 to 10 carbon atoms include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl and the like. Preferred cycloalkyl groups include cyclopentyl and cyclohexyl.

[0049] As used herein, the term "cycloalkenyl" refers to saturated mono- or poly-cyclic hydrocarbon groups containing at least one carbon-carbon double bond. Suitable cycloalkenyl groups having 5 to 10 carbon atoms include, but are not limited to,

cyclopentenyl, 1-methyl-cyclopentenyl, cyclohexenyl, cyclooctenyl, 1,3-cyclopentadienyl, 1,3-hexadienyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, 1,3-cycloheptadienyl, 1,3,5-cycloheptatrienyl and 1,3,5,7-cyclooctatetraenyl. Preferred cycloalkenyl groups include cyclopentenyl and cyclohexenyl.

[0050] The term "aryl" used either alone or in compound words denotes single, polynuclear, conjugated or fused residues of aromatic hydrocarbons. Examples of aryl include, but are not limited to, phenyl, biphenyl, naphthyl, tetrahydronaphthyl. Preferred aryl groups include phenyl and naphthyl.

[0051] The term "heteroaryl" refers to aromatic heterocyclic ring systems, wherein one or more carbon atoms (and where appropriate, hydrogen atoms attached thereto) of a cyclic hydrocarbon residue are replaced with a heteroatom to provide an aromatic residue. Where two or more carbon atoms are replaced, this may be by two or more of the same heteroatom or by different heteroatoms. Suitable heteroatoms include O, N, S and Se. Examples of heteroaryl include, but are not limited to, pyridyl, thienyl, furyl, pyrrolyl, indolyl, pyridazinyl, pyrazolyl, pyrazinyl, thiazolyl, pyimidinyl, quinolinyl, isoquinolinyl, benzofuranyl, benzothienyl, purinyl, quinazolinyl, phenazinyl, acridinyl, benoxazolyl, benzothiazolyl and the like. Preferred heteroaryl groups include pyridyl, thienyl, furyl, pyrrolyl.

[0052] The term "heterocyclyl" when used alone or in compound words includes monocyclic, polycyclic, fused or conjugated hydrocarbon residues, preferably C_{3-10}, preferably C_{3-6}, wherein one or more carbon atoms (and where appropriate, hydrogen atoms attached thereto) are replaced by a heteroatom so as to provide a non-aromatic residue. Suitable heteroatoms include, O, N, S, and Se. Where two or more carbon atoms are replaced, this may be by two or more of the same heteroatom or by different heteroatoms. Suitable examples of heterocyclic groups may include pyrrolidinyl, pyrrolinyl, piperidyl, piperazinyl, morpholino, indolinyl, imidazolidinyl, pyrazolidinyl, thiomorpholino, dioxanyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyrrolyl etc.

[0053] In preferred embodiments, any one of the following may apply:

R_1 is selected from H, an N-terminal capping group that stabilizes the terminus of a helix, usually having hydrogen atoms able to form hydrogen bonds or having a negative charge at the N-terminus to match with the helix dipole, a non-peptidic group or a mimic of an amino acid side chain. Suitable N-terminal capping groups include acyl and N-succinate. Suitable groups that mimic an amino acid side chain are any natural or unnatural amino

acid side chain attached to a carbonyl group derived from a carboxylic acid which has undergone amide bond formation with the N-terminal amino group. Suitable mimics of amino acid side chains include, but are not limited to:

$CH_3CH_2C(O)$-$(CH_2)_u$-, $NH_2(NH{=})CNH$-$C(O)$-$(CH_2)_u$-, $H_2NC(O)(CH_2)_2C(O)$-$(CH_2)_u$-, $HOC(O)(CH_2)_2C(O)$-$(CH_2)_u$-, $HS(CH_2)_2C(O)$-$(CH_2)_u$-, $H_2NC(O)(CH_2)_3C(O)$-$(CH_2)_u$-, $HOC(O)(CH_2)_2C(O)$-$(CH_2)_u$-, (4-imidazolyl)-$(CH_2)C(O)$-$(CH_2)_u$-, $CH_3CH_2CH(CH_3)CH_2C(O)$-$(CH_2)_u$-, $(CH_3)_2CH(CH_2)_2C(O)$-$(CH_2)_u$-, $H_2N(CH_2)_5C(O)$-$(CH_2)_u$-, $CH_3S(CH_2)_3C(O)$-$(CH_2)_u$-, $Ph(CH_2)_2C(O)$-$(CH_2)_u$-, $Ph(CH_2)_4C(O)$-$(CH_2)_u$-, $HO(CH_2)_2C(O)$-$(CH_2)_u$-, $HOCH(CH_3)CH_2C(O)$-$(CH_2)_u$-, (3-indolyl)$(CH_2)_2$-$(CH_2)_u$-, (4-hydroxyphenyl)$(CH_2)_2C(O)$-(CH_2)u-, (4-hydroxyphenyl)$(CH_2)_3C(O)$-$(CH_2)_u$-, $(CH_3)_2CHCH_2C(O)$-$(CH_2)_u$-, $CH_3CH_2CH_2C(O)$-$(CH_2)_u$-, $C_6H_{10}CH_2C(O)$-$(CH_2)_u$-, $C_5H_8CH_2C(O)$-$(CH_2)_u$-, $CH_3C(O)$-$(CH_2)_u$-, $CH_3(CH_2)_4C(O)$-$(CH_2)_u$-, $CH_3(CH_2)_5C(O)$-$(CH_2)_u$-, $HOC(O)CH_2C(O)$-$(CH_2)_u$-, $HS(CH_2)C(O)$-$(CH_2)_u$-, $H_2N(CH_2)_4C(O)$-$(CH_2)_u$- and $HOCH_2C(O)$-$(CH_2)_u$- wherein u is 0 or an integer from 1 to 10. The preferred non-peptidic groups enhance the stability, bioavailability or activity of the peptides. Suitable non-peptidic groups include, but are not limited to hydrophobic groups such as carbobenzoxyl, dansyl, t-butyloxycarbonyl, acetyl, 9-fluorenylmethoxycarbonyl, groups which stabilize or mimic alpha-helices, groups which mimic the secondary structure of peptides, particularly alpha helical peptides, such as those disclosed in WO 03/018587, groups which improve bioavailability, such as hydrophilic groups which aid aqueous solubility, for example, cyclodextrans; groups which are recognised by transport receptors to allow or improve transport of the peptides to the site of activity, for example, transport across cell walls or through an epithelial layer such as skin or the gut wall.

R_2 is selected from H, a C-terminal capping group that stabilizes the terminus of a helix, usually having hydrogen atoms able to form hydrogen bonds or having a positive charge at the C-terminus to match with the helix dipole, a peptide of 1, 2, 3, 4 or 5 amino acid residues optionally capped with a C-terminal capping group that stabilizes the terminus of a helix, usually having hydrogen atoms able to form hydrogen bonds or having a positive charge at the C-terminus to match with the helix dipole, a mimic of an amino acid side chain or a group which activates the terminal carboxylic acid carbonyl group to nucleophilic substitution. A suitable C-terminal capping group is NH_2. Suitable mimics of amino acid side chains are any common or unnatural amino acid side chain attached to an amine group which forms an amide bond with the C-terminal carboxylic acid. Suitable

mimics of amino acid side chains include but are not limited to:

$-(CH_2)_u-NHCH_2CH_3$, $-(CH_2)_u-NH(CH_2)_4NHC(=NH)NH_2$, $-(CH_2)_u-NH(CH_2)_2C(O)-NH_2$, $-(CH_2)_u-NH-(CH_2)_2CO_2H$, $-(CH_2)_u-NH-(CH_2)_2-SH$, $-(CH_2)_u-NH(CH_2)_3C(O)NH_2$, $-(CH_2)_u-NH(CH_2)_3-CO_2H$, $-(CH_2)_u-NH-(CH_2)_2$(4-imidazolyl), $-(CH_2)_u-NH-CH_2CH(CH_3)CH_2CH_3$, $-(CH_2)_u-NH-(CH_2)_2CH(CH_3)_2$, $-(CH_2)_u-NH-(CH_2)_5NH_2$, $-(CH_2)_u-NH-(CH_3)_3SCH_3$, $-(CH_2)_u-NH-(CH_2)_2$(3-indolyl), $-(CH_2)_u-NH-(CH_2)_2$(4-hydroxyphenyl), $-(CH_2)_u-NH(CH_2)_3$(4-hydroxyphenyl), $-(CH_2)_u-NH-CH_2CH(CH_3)_2$, $-(CH_2)_u-NHCH_2CH_2CH_3$, $-(CH_2)_u-NH-CH_2C_6H_{10}$, $-(CH_2)_u-NH-CH_2C_5H_8$, $-(CH_2)_u-NHCH_3$, $-(CH_2)_u-NH(CH_2)_4CH_3$, $-(CH_2)_u-NH(CH_2)_5CH_3$, $-(CH_2)_u-NHCH_2CO_2H$, $-(CH_2)_u-NHCH_2-SH$, $-(CH_2)_u-NH-(CH_2)_2OH$, $-(CH_2)_u-NH-(CH_2)_5NH_2$ and $-(CH_2)_u-NH-CH_2OH$; wherein u is 0 or an integer from 1 to 10.

Suitable groups which activate the C-terminal carboxylic to nucleophilic attack include converting the carboxylic acid to an acid chloride, an acid anhydride, an acyl azide, an O-acylisourea, a phosphonium derivative or an activated ester, especially those known in the art for activating carboxylic acids for peptide bond formation.

The preferred non-peptidic groups enhance the stability, bioavailability or activity of the peptides. Suitable non-peptidic groups include but are not limited to hydrophobic groups such as t-butyl, groups which stabilize or mimic alpha-helices, groups which mimic the secondary structure of peptides, particularly alpha helical peptides, such as those disclosed in WO 03/018587, groups which improve bioavailability, such as hydrophilic groups which aid aqueous solubility, for example, cyclodextrans; groups which are recognised by transport receptors to allow or improve transport of the peptides to the site of activity, for example, transport across cell walls or through an epithelial layer such as skin or the gut wall.

Each R′ is selected from H, CH_3, CH_2CH_3, vinyl, OH, OCH_3, NH_2, $NH(CH_3)$, $N(CH_3)_2$, phenyl, F or Cl; most preferably H or CH_3, especially H.

Each R″ is selected from H, CH_3, CH_2CH_3 or vinyl, especially H.

m is 1 and n is 3 or 4, m is 2 and n is 4, m is 3 and n is 1 or m is 4 and n is 1 or 2, especially where m is 1 and n is 4.

Each Xaa may be any amino acid residue selected to mimic the amino acid residues in a known alpha helical peptide of interest or to prepare an unknown peptide having new properties. An individual Xaa can be the same or different as another Xaa and is preferably selected from a D- or L- alpha amino acid residue. Especially preferred peptides of formula

(I) have at least one Xaa which is a D- or L- alpha amino acid residue that is favourable to helix formation. Even more preferred are peptides in which 2 or 3 of Xaa are D- or L- alpha amino acid residues that are favourable to helix formation, for example, alanine, arginine, lysine, methionine, leucine, glutamic acid, glutamine, cysteine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine and aspartic acid, especially alanine, arginine, lysine, methionine, leucine, glutamic acid and glutamine.

L is preferably –NH-C(O)- or –C(O)-NH-.

[0054] Surprisingly, the cyclic pentapeptides of the invention are tolerant of variation of Xaa residues, with little effect on alpha helicity of the peptide.

[0055] Representative peptides of the invention include, but are not limited to:

cyclo-1,5-Ac[KXaaXaaXaaD]-NH_2	SEQ ID NO. 1
cyclo-1,5-Ac[DXaaXaaXaaK]-NH_2	SEQ ID NO. 2
cyclo-1,5-Ac[KXaaXaaXaaE]-NH_2	SEQ ID NO. 3
cyclo-1,5-Ac[EXaaXaaXaaK]-NH_2	SEQ ID NO. 4
cyclo-1,5-Ac[OXaaXaaXaaD]-NH_2	SEQ ID NO. 5
cyclo-1,5-Ac[DXaaXaaXaaO]-NH_2	SEQ ID NO. 6
cyclo-2,6-AcXaa[KXaaXaaXaaD]-NH_2	SEQ ID NO. 7

[0056] Especially preferred peptides are those of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6 and SEQ ID NO. 7, more especially SEQ ID NO. 1 and SEQ ID NO. 7.

[0057] Illustrative examples of amino acid sequences represented by the above peptides include:

cyclo-1,5-Ac[KARAD]-NH_2	SEQ ID NO. 8
cyclo-1,5-Ac[DARAK]-NH_2	SEQ ID NO. 9
cyclo-1,5-Ac[KARAE]-NH_2	SEQ ID NO. 10
cyclo-1,5-Ac[EARAK]-NH_2	SEQ ID NO. 11
cyclo-1,5-Ac[OARAD]-NH_2	SEQ ID NO. 12
cyclo-1,5-Ac[DARAO]-NH_2	SEQ ID NO. 13
cyclo-2,6-AcR[KSSSD]-NH_2	SEQ ID NO. 14

cyclo-2,6-AcR[KGGGD]-NH_2 SEQ ID NO. 15

cyclo-2,6-AcR[KAAAD]-NH_2 SEQ ID NO. 16

cyclo-2,6-AcR[KGSAD]-NH_2 SEQ ID NO. 17

[0058] In another aspect, the present invention provides a method for constructing a constrained helical peptide comprising the steps of: (1) synthesising a peptide, wherein the peptide comprises a sequence of five amino acid residues having a first terminal residue and a second terminal residue that are separated by an intervening sequence of three amino acid residues, and wherein the individual side chains of the first and second terminal residues are linkable to each other; and (2) cyclising the peptide by linking the side chain of the first terminal residue with the side chain of the second terminal residue, thereby yielding a constrained helical peptide. In certain embodiments, the first terminal residue has a side chain containing an amide bond-forming substituent and the second terminal residue has a side chain containing a functional group capable of forming an amide linkage with the side chain amide bond-forming substituent of the first terminal residue and the peptide is cyclised by reacting the side chain amide bond-forming substituent of the first terminal residue with the functional group of the second terminal residue to form an amide bond linkage, thereby yielding a constrained helical peptide. During peptide synthesis, reactive groups on the side chains, including the amide forming substituents are suitably protected, for example, carboxy groups can be suitably protected as esters such as methyl, ethyl, allyl, benzyl, t-butyl or phenyl esters and amino groups can be suitably protected with alkyloxy carbonyl, allyloxycarbonyl (Alloc)benzyloxycarbonyl (Z), t-butoxycarbonyl (Boc), 2-(4-biphenylyl)-isopropoxycarbonyl (Bpoc), 9-fluorenylmethoxycarbonyl (Fmoc), triphenylmethyl (trityl) or 2-nitrophenylsulphenyl (Nps) groups, which may be removed after synthesis of the peptide and before reaction to form the amide bond linkage. Suitable methods for selectively protecting and deprotecting functional groups can be found in Green & Wutz[94] and Taylor (2002)[43].

[0059] The peptides of the present invention may be prepared using techniques known in the art. For example, peptides can be synthesised using various solid phase techniques[91] or using an automated synthesis and standard F-moc chemistry[92]. These techniques are also suitable for incorporating non-naturally occurring amino acid residues into the amino acid sequence.

[0060] Alternatively, non-naturally occurring amino acids may be incorporated into the sequence by manipulation of a residue in the sequence. For example, the hydroxy

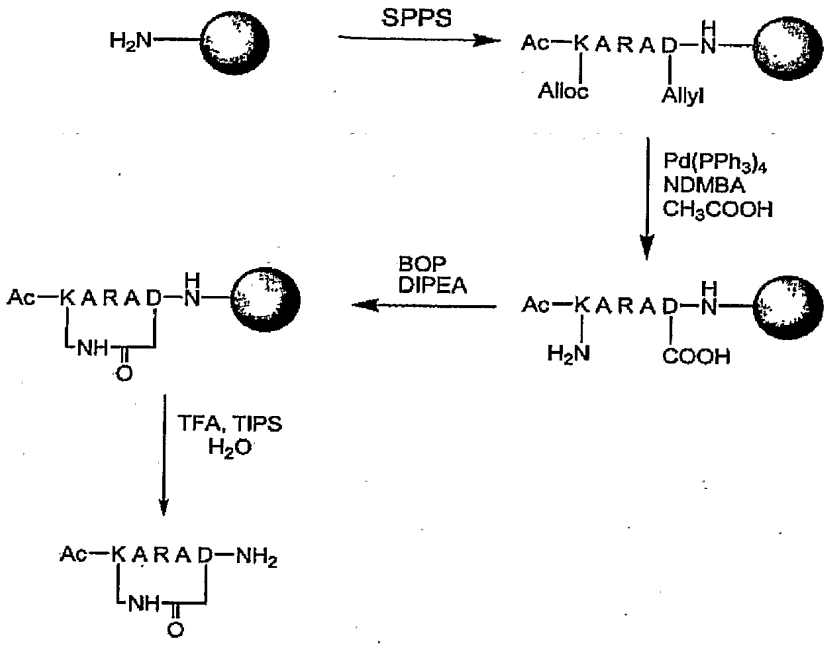
group or thiol group of threonine, serine or cysteine may be alkylated to provide an ether or thioether, or substituents may be introduced into the phenyl ring of phenylalanine or tyrosine using known substitution reactions such as Friedel-Crafts alkylation or acylation.

[0061] Once the peptides of the present invention have been prepared, they may be substantially purified using preparative HPLC. The composition of the peptides can be confirmed by amino acid analysis or by sequencing, for example, using the Edman Degradation procedure.

[0062] Suitable protecting groups for use during solid phase synthesis or solution phase of the amino acid sequences, together with suitable protecting and deprotecting methods for reactive functional groups such as amines and carboxylic acids, are known in the art, for example, as found in Green & Wutz[94].

[0063] Once the peptide is prepared and deprotection of the side chains is effected, cyclization to form a cyclic peptide may be achieved by methods known in the art. For example, an amide bond may be formed between a side chain carboxylic acid and a side chain amine by activation of the carboxylic acid, for example, as an acid chloride, acid anhydride, an acyl azide, a carbodiimide, an acyloxyphosphonium or uronium compound or an active ester, and allowing nucleophilic attack from the amine nitrogen atom. A particularly preferred method of activating the carboxylic acid to nucleophilic attack is preparation of an acyloxyphosphonium or uronium derivative of the carboxylic acid, for example, by reaction with the carboxylic acid with benzotriazolyloxy-tri-(dimethylamino)phosphonium hexafluorophosphate (BOP) or benzotriazolyloxy-tris-(pyrrolidinyl)phosphonium hexafluorophosphate (Py-BOP) in the presence of a tertiary amine such as triethylamine or diisopropylethylamine or similar reaction using Benzotriazol-1-yl-1,1,3,3-tetramethyluronium ion (HBTU).

[0064] A representative solid phase synthesis is shown in Scheme 1:



Scheme 1

[0065] The peptides of the invention are designed to mimic binding determinants from α-helical binding domains of known proteins. Such peptides have a number of uses, including the determination of whether a binding determinant in an α-helical binding domain of a known protein can serve as a structural model for the design of peptidomimetics or small molecules capable of mimicking or antagonising the binding activity of the intact protein. In using the peptides of the invention for this purpose, the practitioner may select a binding protein with an α-helical domain that interacts with a ligand, and then identify a candidate binding determinant situated within a sequence of (e.g., three or more) contiguous amino acid residues in the helical binding domain. The candidate binding determinant can be identified by using mutagenesis (e.g., alanine scanning mutagenesis) to determine whether the candidate sequence contains one or more amino acid residues that are critical for ligand binding. Subsequently, a constrained peptide containing the candidate sequence is designed by selecting two residues in the candidate sequence (designated *i* and *i*+4) which are separated by an intervening sequence of *n-1* (e.g., 3) amino acid residues and which do not interact with ligand (as determined by mutagenesis in the previous step) for substitution with amino acid residues having side chains that can be linked to each other. The peptide is synthesised and the side chains of the foreign *i* and *i*+4 residues are used to tether the peptide in an α-helical conformation according to the methods of the invention described herein. Finally, the peptide's binding

activity with the ligand is assayed, e.g., in a binding competition assay with the intact binding protein, and the results of the assay can be used to determine whether a peptidomimetic or small molecule antagonist could be developed using the binding determinant as a structural model.

[0066] Thus, in a further aspect, the invention contemplates the use an alpha helical cyclic peptide, wherein the peptide comprises a sequence of five amino acid residues having a first terminal residue and a second terminal residue that are separated by an intervening sequence of three amino acid residues, and wherein the side chains of the first and second terminal residues are linked to each other as a scaffold for presenting the side chains of at least some of the five amino acid residues in a (three dimensional) conformation that is analogous to the conformation of amino acid side chains of at least a portion of an α-helical domain of a known alpha protein. In some embodiments, the side chains of at least 1 or 2 or all 3 of the intervening amino acid residues are so analogously presented. In other embodiments, the side chains of at least 1 or 2 or all 3 of the intervening amino acid residues and at least one terminal amino acid residue are so analogously presented. Suitably, at least part of the conformationally constrained secondary structure defined by the five amino acid residues (i.e., pentapeptide) mimics a member of a ligand-receptor binding pair. Illustrative examples of ligand-receptor binding pairs include protein-DNA binding partners (e.g., Zif268 and G/C rich major groove), protein-RNA binding partners (e.g., HIV reverse transcriptase and Rev response element (RRE); λ–N peptide and BoxB RNA; p22 peptides and BoxB RNA) and protein-protein binding partners (e.g., p53 and HDM2; Bak and Bcl-X_L; VHL peptide and Elongin C; VP16 activation domain and $HTAF_{II}31$; hPTH and hPTHrP; Dynorphin A and κ,δ-Opioid receptors; Apolipoprotein-E and LDL receptor; Neuropeptide-Y and NPY receptors; Galanin and Gal receptors; Corticotropin Releasing Factor and CRF receptors; Calcitonin Gene Related Peptide and CGRP receptors; Nociceptin and ORL1 receptor; Vasointestinal Peptide and $VPAC_{1\ \&\ 2}$; and Nuclear Coactivators (eg. SRC1, GRIP1) and Nuclear Receptors.

[0067] While not wishing to be limited by any one particular theory or mode of operation, the constrained helical peptides of the present invention are believed to derive their activity by interaction of the $i \rightarrow i+4$ face of the helix. However, when two or more tandemly arrayed constrained helical peptides are present, as part of an extended helix polypeptide backbone or super helix, the positions $i \rightarrow i+4$ of a first constrained helical

pentapeptide will be offset by approximately one third of a turn relative to positions $i \rightarrow i+4$ of a second constrained helical pentapeptide. In other words, the $i \rightarrow i+4$ faces of the two helices will not be aligned directly in the same plane and will be out of register by approximately one third of a turn. Thus, in certain embodiments where an extended helix polypeptide backbone or super helix is required for interaction with a biomolecule of interest, it may be desirable to take this offset into account when designing a helical peptide so that one face of its helix is substantially free of any cyclizing linkages that may occlude or otherwise interfere with this interaction. In illustrative examples, the helical peptide may simply comprise two or three consecutive constrained helical pentapeptides. In other illustrative examples, the helical peptide may comprise two consecutive constrained helical pentapeptides spaced from a third constrained helical pentapeptide by about 1, 2, 5, 8 or 9 natural or unnatural helix-forming amino acid residues. In still other illustrative examples, the helical peptide may comprise three consecutive constrained helical pentapeptides spaced from a fourth constrained helical pentapeptide by about 0, 3, 4, 6 or 7 natural or unnatural helix-forming amino acid residues; or alternatively 1, 2, 5, 6 or 9 natural or unnatural helix-forming amino acid residues, depending on which face is required to be kept substantially free of any cyclizing linkages. In still other illustrative examples, the helical peptide may comprise four consecutive constrained helical pentapeptides spaced from a fifth constrained helical pentapeptide by about 1, 2 or 3 natural or unnatural helix-forming amino acid residues. In still other illustrative examples, the helical peptide may comprise five consecutive constrained helical pentapeptides spaced from a sixth constrained helical pentapeptide by about 2, 7, 12 or 17 natural or unnatural helix-forming amino acid residues. The optimal spacing between cyclic pentapeptide modules is determined on a case-by-case basis and would be readily apparent to a person skilled in the art through simple molecular modelling experiments using commercially available programs (e.g., InsightII)[104].

[0068] In certain embodiments which require mimicking multiple turns of an α-helical binding domain, the conformationally constrained peptide comprises a plurality (e.g., 2, 3, 4, 5, 6, 7, 8, 9 or more) of pentapeptides as broadly described above. Accordingly, in yet another aspect, the present invention provides the use of a conformationally constrained peptide having a plurality of alpha helical pentapeptide sequences, wherein the pentapeptide sequences comprise a sequence of five amino acid residues having a first terminal residue and a second terminal residue that are separated by an intervening sequence of three amino acid residues, and wherein the side chains of the first and second

terminal residues are linked to each other, as a scaffold for presenting the side chains of at least some of the amino acid residues of the pentapeptide sequences in a (three-dimensional) configuration that is analogous to the configuration of amino acid side chains of at least a portion of an α-helical domain of a known protein.

[0069] As used herein, the term "scaffold" is used in its broadest sense and includes a region or domain that has a conserved tertiary structural motif that can be modified to display one or more specific amino acid residues in a fixed conformation.

[0070] In some embodiments, the side chains of at least 1 or 2 or all 3 of the intervening amino acid residues of each pentapeptide sequence are so analogously presented. In other embodiments, the side chains of at least 1 or 2 or all 3 of the intervening amino acid residues and at least one terminal amino acid residue of each pentapeptide sequence are so analogously presented. Suitably, at least part of the conformationally constrained secondary structure defined by the pentapeptide sequences mimics a member of a ligand-receptor binding pair. In illustrative examples, some or all of the pentapeptides are located adjacent to each other. Alternatively, at least one of the pentapeptides is spaced from a pair of adjacent pentapeptides.

[0071] In certain embodiments, the conformationally constrained peptides of the invention are designed to mimic epitopes in proteins and are used to selectively raise polyclonal or monoclonal antibodies against such individual epitopes. Since the peptides will frequently be too small to generate an immune response, the peptides can be conjugated to carriers known to be immunogenic in the species to be immunised, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatising agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or R^1 N=C=NR, where R and R^1 are different alkyl groups.

[0072] Advantageously, the macrocyclic moiety of the pentapeptide is stable in water to temperatures of up to about 80°C and stable to denaturants such as 8M guanidine hydrochloride, and to the degradative effects of proteolytic enzymes such as trypsin or human serum. The alpha helical short-chain peptides are therefore suitable for use as chemical or biological probes, pharmaceuticals, biotechnology products such as vaccines or diagnostic agents, new components of novel biopolymers and as industrial agents.

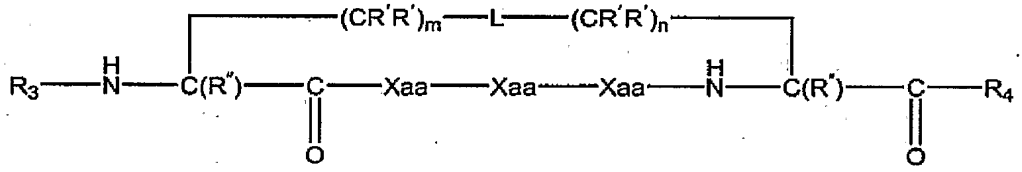
[0073] The alpha helical pentapeptides of the invention can be used alone to mimic a specific peptide motif of a protein or polypeptide or may be incorporated into a larger polymeric or non polymeric non-peptidic molecules.

[0074] In another aspect of the present invention there is provided a use of at least one alpha helical cyclic peptide, wherein the peptide comprises a sequence of five amino acid residues having a first terminal residue and a second terminal residue that are separated by an intervening sequence of three amino acid residues, and wherein the side chains of the first and second terminal residues are linked to each other, as a macrocyclic module for incorporation into a non-peptidic molecular structure, or for constructing a multi-macrocyclic structure that mimics multiple turns of an alpha helix.

[0075] Multi-macrocyclic structures may provide new or unknown three dimensional positioning of side chains in an alpha helix or may mimic a portion of, or an entire, alpha helical motif from a known protein or polypeptide.

[0076] In a preferred embodiment, the alpha helical cyclic peptide, be used as the scaffold or macrocyclic module has the formula (II):

(II)



wherein each Xaa is independently selected from any amino acid;

each R′ and R″ are independently selected from H, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10}cylcoalkyl, C_5-C_{10}cycloalkenyl, -OH, -OC_1-C_{10} alkyl, -NH_2, -NH(C_1-C_{10}alkyl), -N(C_1-C_{10}alkyl)$_2$, C_6-C_{10}aryl, C_3-C_{10}heterocyclyl, C_5-C_{10}heteroaryl and halo;

L is selected from –NH-C(O)-, -C(O)-NH-, -S-S-, -CH(OH)CH_2-, CH_2CH(OH)-, -CH=CH-, -CH_2-CH_2-, -NH-CH_2- -CH_2-NH-, -CH_2-S-, -S-CH_2-, -C(O)-CH_2-, -CH_2-C(O)-, -S(O)$_t$-NH-, -NH-S(O)$_t$-, CH_2-P(=O)(OH)- and –P(=O)(OH)-CH_2-;

R_3 is selected from H, an N-capping group or a mimic of an amino acid side chain,

R_4 is selected from H, a C-terminal capping group, a mimic of an amino acid side chain or

a group which activates the terminal carboxylic acid carbonyl group to nucleophilic substitution;

m is an integer from 1 to 4 and

n is an integer from 1 to 4,

wherein m + n = 4, 5 or 6 and wherein when m is 2, n is not 3 and when m is 3, n is not 2.

[0077] In preferred embodiments, any one of the following may apply:

R_1 is selected from H, an N-terminal capping group that stabilizes the terminus of a helix, usually having hydrogen atoms able to form hydrogen bonds or having a negative charge at the N-terminus to match with the helix dipole, or a mimic of an amino acid side chain. Suitable N-terminal capping groups include acyl and N-succinate. Suitable groups that mimic an amino acid side chain are any natural or unnatural amino acid side chain attached to a carbonyl group derived from a carboxylic acid which has undergone amide bond formation with the N-terminal amino group. Suitable mimics of amino acid side chains include, but are not limited to:

$CH_3CH_2C(O)$-$(CH_2)_u$-, $NH_2(NH{=})CNH$-$C(O)$-$(CH_2)_u$-, $H_2NC(O)(CH_2)_2C(O)$-$(CH_2)_u$-, $HOC(O)(CH_2)_2C(O)$-$(CH_2)_u$-, $HS(CH_2)_2C(O)$-$(CH_2)_u$-, $H_2NC(O)(CH_2)_3C(O)$-$(CH_2)_u$-, $HOC(O)(CH_2)_2C(O)$-$(CH_2)_u$-, (4-imidazolyl)-$(CH_2)C(O)$-$(CH_2)_u$-, $CH_3CH_2CH(CH_3)CH_2C(O)$-$(CH_2)_u$-, $(CH_3)_2CH(CH_2)_2C(O)$-$(CH_2)_u$-, $H_2N(CH_2)_5C(O)$-$(CH_2)_u$-, $CH_3S(CH_2)_3C(O)$-$(CH_2)_u$-, $Ph(CH_2)_2C(O)$-$(CH_2)_u$-, $Ph(CH_2)_4C(O)$-$(CH_2)_u$-, $HO(CH_2)_2C(O)$-$(CH_2)_u$-, $HOCH(CH_3)CH_2C(O)$-$(CH_2)_u$-, (3-indolyl)$(CH_2)_2$-$(CH_2)_u$-, (4-hydroxyphenyl)$(CH_2)_2C(O)$-(CH_2)u-, (4-hydroxyphenyl)$(CH_2)_3C(O)$-$(CH_2)_u$-, $(CH_3)_2CHCH_2C(O)$-$(CH_2)_u$-, $CH_3CH_2CH_2C(O)$-$(CH_2)_u$-, $C_6H_{10}CH_2C(O)$-$(CH_2)_u$-, $C_5H_8CH_2C(O)$-$(CH_2)_u$-, $CH_3C(O)$-$(CH_2)_u$-, $CH_3(CH_2)_4C(O)$-$(CH_2)_u$-, $CH_3(CH_2)_5C(O)$-$(CH_2)_u$-, $HOC(O)CH_2C(O)$-$(CH_2)_u$-, $HS(CH_2)C(O)$-$(CH_2)_u$-, $H_2N(CH_2)_4C(O)$-$(CH_2)_u$- and $HOCH_2C(O)$-$(CH_2)_u$- wherein u is 0 or an integer from 1 to 10;

R_2 is selected from H, a C-terminal capping group that stabilizes the terminus of a helix, usually having hydrogen atoms able to form hydrogen bonds or having a positive charge at the C-terminus to match with the helix dipole, a mimic of an amino acid side chain or a group which activates the terminal carboxylic acid carbonyl group to nucleophilic substitution. A suitable C-terminal capping group is NH_2. Suitable mimics of amino acid side chains are any common or unnatural amino acid side chain attached to an amine group which forms an amide bond with the C-terminal carboxylic acid. Suitable mimics of amino

acid side chains include but are not limited to:

$-(CH_2)_u-NHCH_2CH_3$, $-(CH_2)_u-NH(CH_2)_4NHC(=NH)NH_2$, $-(CH_2)_u-NH(CH_2)_2C(O)-NH_2$, $-(CH_2)_u-NH-(CH_2)_2CO_2H$, $-(CH_2)_u-NH-(CH_2)_2-SH$, $-(CH_2)_u-NH(CH_2)_3C(O)NH_2$, $-(CH_2)_u-NH(CH_2)_3-CO_2H$, $-(CH_2)_u-NH-(CH_2)_2$(4-imidazolyl), $-(CH_2)_u-NH-CH_2CH(CH_3)CH_2CH_3$, $-(CH_2)_u-NH-(CH_2)_2CH(CH_3)_2$, $-(CH_2)_u-NH-(CH_2)_5NH_2$, $-(CH_2)_u-NH-(CH_3)_3SCH_3$, $-(CH_2)_u-NH-(CH_2)_2$(3-indolyl), $-(CH_2)_u-NH-(CH_2)_2$(4-hydroxyphenyl), $-(CH_2)_u-NH(CH_2)_3$(4-hydroxyphenyl), $-(CH_2)_u-NH-CH_2CH(CH_3)_2$, $-(CH_2)_u-NHCH_2CH_2CH_3$, $-(CH_2)_u-NH-CH_2C_6H_{10}$, $-(CH_2)_u-NH-CH_2C_5H_8$, $-(CH_2)_u-NHCH_3$, $-(CH_2)_u-NH(CH_2)_4CH_3$, $-(CH_2)_u-NH(CH_2)_5CH_3$, $-(CH_2)_u-NHCH_2CO_2H$, $-(CH_2)_u-NHCH_2-SH$, $-(CH_2)_u-NH-(CH_2)_2OH$, $-(CH_2)_u-NH-(CH_2)_5NH_2$ and $-(CH_2)_u-NH-CH_2OH$; wherein u is 0 or an integer from 1 to 10.

Suitable groups which activate the C-terminal carboxylic to nucleophilic attack include converting the carboxylic acid to an acid chloride, an acid anhydride, an acyl azide, an O-acylisourea, a phosphonium derivative or an activated ester, especially those known in the art for activating carboxylic acids for peptide bond formation;

Each R′ is selected from H, CH_3, CH_2CH_3, vinyl, OH, OCH_3, NH_2, $NH(CH_3)$, $N(CH_3)_2$, phenyl, F or Cl; most preferably H or CH_3, especially H;

Each R″ is selected from H, CH_3, CH_2CH_3 or vinyl, especially H;

m is 1 and n is 3 or 4, m is 2 and n is 4, m is 3 and n is 1 or m is 4 and n is 1 or 2, especially where m is 1 and n is 4;

Each Xaa may be any amino acid residue selected to mimic the amino acid residues in a known alpha helical peptide of interest or to prepare an unknown peptide having new properties. Xaa is preferably a D- or L- alpha amino acid residue. Especially preferred peptides of formula (I) have at least one Xaa which is a D- or L- alpha amino acid residue that is favourable to helix formation. Even more preferred are peptides in which 2 or 3 of Xaa are D- or L- alpha amino acid residues that are favourable to helix formation, for example, alanine, arginine, lysine, methionine, leucine, glutamic acid, glutamine, cysteine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine and aspartic acid, especially alanine, arginine, lysine, methionine, leucine, glutamic acid and glutamine; and

L is selected from –NH-C(O)- and –C(O)-NH-.

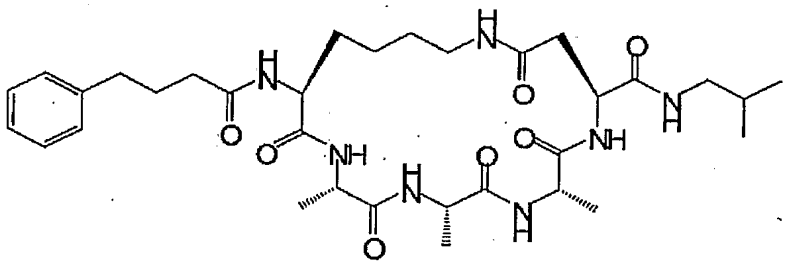
[0078] Scaffolds or macrocyclic modules of formula (II) can be prepared as described for peptides of formula (I).

[0079] N-terminal capping groups may and groups which mimic an amino acid side chain may be introduced by methods known in the art. For example the N-terminal amino group may be reacted with a carboxylic acid derivative of the capping group or mimic or an activated carboxylic acid derivative to form an amide bond.

[0080] C-terminal capping groups and groups which mimic an amino acid side chain may be introduced by methods known in the art. For example the C-terminal carboxylic acid may be activated and reacted with an amine derivative, preferably a primary amine derivative of the C-terminal capping group or group that mimics an amino acid side chain.

[0081] C-terminal carboxylic acid groups or any other carboxylic acid groups that require activation toward nucleophilic substitution can be activated by methods known in the art[95]. For example the carboxylic acid may be activated by conversion to an acyl chloride using PCl_5 or $SOCl_2$, conversion to an acyl azide by hydrazinolysis of a protected amino acid or peptide ester followed by treatment with $NaNO_2$ in aqueous acid, conversion to a symmetrical or mixed anhydride using two equivalents of an amino acid and a dicyclohexylcarbodiimide or by reaction with an acid chloride in a dry solvent in the presence of a mild base, conversion to an O-acylisourea by reaction with dicyclohexylcarbodiimide or by conversion to an acyloxyphosphonium or uronium species by reacting a carboxylate anion with a phosphonium or uronium cation, for example, BOP, PyBOP or HBTU.

[0082] A representative example of an alpha helical pentapeptide as a scaffold for projecting attached substituents into positions normally occupied the side chains of longer peptides than pentapeptides is given by formula (III):



(III)

[0083] The pentapeptide of formula (III) is an example of a peptide of formula (II) in which the three variable amino acid residues that represent Xaa are all alanine, the

macrocycle is formed by amide bond formation between a lysine residue (Y) and an aspartic acid residue (Z), R_3 is an amide formed from the reaction of phenylbutanoic acid and the N-terminal amino group and mimics a phenylalanine side chain, and R_4 is an amide formed by the reaction of isobutyl amine with an activated C-terminal carboxylic acid and mimics a valine side chain.

[0084] The scaffold or macrocyclic module may also be incorporated into a multi-macrocyclic structure or may be incorporated into a non-peptidic molecule.

[0085] As used herein, the term "macrocyclic module" refers to a cyclic pentapeptide which may be unsubstituted at the N and C termini or may be activated for incorporation into a larger structure. For example, a pentacyclic peptide of formula II in which R_3 is H and R_4 is H or a group which activates the terminal carboxylic acid carbonyl group to nucleophilic substitution is a macrocyclic module.

[0086] Preparation of a non-peptidic molecule incorporating a scaffold or macrocyclic module may be prepared by reacting the N-terminal and/or activated C-terminal of the macrocyclic module with desired non-peptidic moieties.

[0087] Alternatively, a number of modules, which may be the same or different, may be prepared as described herein and then consecutively linked to form a multi-macrocyclic peptide that mimics a number of turns of an alpha helix. The multi-macrocyclic peptide may then be used to mimic a protein or polypeptide or part thereof, or may be incorporated into a longer peptide sequence.

[0088] Accordingly, in a further aspect of the invention there is provided a conformationally constrained peptide having a plurality of alpha helical pentapeptide sequences, wherein the pentapeptide sequences comprise a sequence of five amino acid residues having a first terminal residue and a second terminal residue that are separated by an intervening sequence of three amino acid residues, and wherein the side chains of the first and second terminal residues are linked to each other.

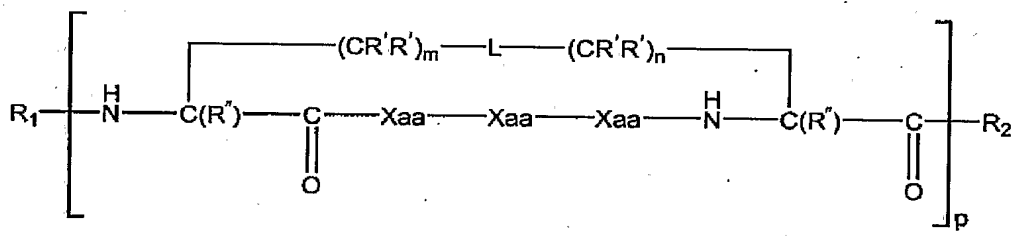
[0089] In a preferred embodiment at least one of the alpha helical pentapeptide sequences is a pentapeptide module of formula (II).

[0090] The number of macrocyclic modules in the peptide or polypeptide will depend on the length of the alpha helical portion of the polypeptide required. If the peptide is intended to mimic an alpha helical portion of a known protein or polypeptide, the number of macrocyclic modules will be determined by the number of turns in the alpha helical

portion of the known protein or polypeptide. For example, two cyclic pentapeptide modules of Formula (II) could be linked such that Z is directly bonded to Y, to form a 2.8-turn alpha helix. In a similar manner, three consecutively linked cyclic pentapeptide modules would form a 4.2-turn alpha helix, four consecutively linked cyclic pentapeptide modules would form a 5.6-turn alpha helix, five consecutively linked cyclic pentapeptide modules would form a 6.9-turn alpha helix, six consecutively linked cyclic pentapeptide modules would form a 8.3-turn alpha helix and larger alpha helices may be obtained in a similar fashion. In this manner multi-macrocyclic assemblies which are alpha helical in nature can be obtained.

[0091] In a preferred embodiment the conformationally constrained peptide having a plurality of alpha helical pentapeptide sequences, is a compound of formula (IV):

(IV)



wherein each Xaa is independently selected from any amino acid residue;

wherein each Xaa is independently selected from any amino acid residue;

R_1 is selected from H, an N-terminal capping group, a peptide of 1 to 20 amino acid residues optionally capped by an N-terminal capping group, a non-peptidic group or a group that mimics an amino acid side chain;

R_2 is selected from H, a C-terminal capping group, a peptide of 1 to 20 amino acids optionally capped by a C-terminal capping group, a group that mimics an amino acid side chain or a group that activates the terminal carboxylic acid carbonyl group to nucleophilic substitution;

each R′ and R″ are independently selected from H, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10}cylcoalkyl, C_5-C_{10}cycloalkenyl, -OH, -OC_1-C_{10} alkyl, -NH_2, -NH(C_1-C_{10}alkyl), -N(C_1-C_{10}alkyl)$_2$, C_6-C_{10}aryl, C_3-C_{10}heterocyclyl, C_5-C_{10}heteroaryl and halo;

L is selected from –NH-C(O)-, –C(O)-NH-, -S-S-, -CH(OH)CH_2-, CH_2CH(OH)-, -CH=CH-, -CH_2-CH_2-, -NH-CH_2- -CH_2-NH-, -CH_2-S-, -S-CH_2-, -C(O)-CH_2-, -CH_2-C(O)-, -$S(O)_t$-NH-, -NH-$S(O)_t$-, CH_2-P(=O)(OH)- and –P(=O)(OH)-CH_2-;

m is an integer from 1 to 4 and

n is an integer from 1 to 4

wherein m + n = 4, 5 or 6 and wherein when m is 2, n is not 3 and when m is 3, n is not 2; and

p is an integer from 2 to 12; with the proviso that bicyclo (Lys^{13} – Asp^{17}, Lys^{18} – Asp^{22}) [Ala^1, Nlc^8, Lys^{18}, Asp^{22}, Leu^{27}] hPTH (1-31) NH_2 is excluded.

[0092] In preferred embodiments, any one of the following may apply:

R_1 is selected from H, an N-terminal capping group that stabilizes the terminus of a helix, usually having hydrogen atoms able to form hydrogen bonds or having a negative charge at the N-terminus to match with the helix dipole, a peptide of 1 to 15, 1 to 10 or 1 to 5 amino acid residues optionally capped with an N-terminal capping group that stabilizes the terminus of a helix, usually having hydrogen atoms able to form hydrogen bonds or having a negative charge at the N-terminus to match with the helix dipole, or a mimic of an amino acid side chain. Suitable N-terminal capping groups include acyl and N-succinate. Suitable groups that mimic an amino acid side chain are any natural or unnatural amino acid side chain attached to a carbonyl group derived from a carboxylic acid which has undergone amide bond formation with the N-terminal amino group. Suitable mimics of amino acid side chains include, but are not limited to:

$CH_3CH_2C(O)$-$(CH_2)_u$-, $NH_2(NH=)CNH$-C(O)-$(CH_2)_u$-, $H_2NC(O)(CH_2)_2C(O)$-$(CH_2)_u$-, $HOC(O)(CH_2)_2C(O)$-$(CH_2)_u$-, $HS(CH_2)_2C(O)$-$(CH_2)_u$-, $H_2NC(O)(CH_2)_3C(O)$-$(CH_2)_u$-, $HOC(O)(CH_2)_2C(O)$-$(CH_2)_u$-, (4-imidazolyl)-$(CH_2)C(O)$-$(CH_2)_u$-, $CH_3CH_2CH(CH_3)CH_2C(O)$-$(CH_2)_u$-, $(CH_3)_2CH(CH_2)_2C(O)$-$(CH_2)_u$-, $H_2N(CH_2)_5C(O)$-$(CH_2)_u$-, $CH_3S(CH_2)_3C(O)$-$(CH_2)_u$-, $Ph(CH_2)_2C(O)$-$(CH_2)_u$-, $Ph(CH_2)_4C(O)$-$(CH_2)_u$-, $HO(CH_2)_2C(O)$-$(CH_2)_u$-, $HOCH(CH_3)CH_2C(O)$-$(CH_2)_u$-, (3-indolyl)$(CH_2)_2$-$(CH_2)_u$-, (4-hydroxyphenyl)$(CH_2)_2C(O)$-(CH_2)u-, (4-hydroxyphenyl)$(CH_2)_3C(O)$-$(CH_2)_u$-, $(CH_3)_2CHCH_2C(O)$-$(CH_2)_u$-, $CH_3CH_2CH_2C(O)$-$(CH_2)_u$-, $C_6H_{10}CH_2C(O)$-$(CH_2)_u$-, $C_5H_8CH_2C(O)$-$(CH_2)_u$-, $CH_3C(O)$-$(CH_2)_u$-, $CH_3(CH_2)_4C(O)$-$(CH_2)_u$-, $CH_3(CH_2)_5C(O)$-$(CH_2)_u$-, $HOC(O)CH_2C(O)$-$(CH_2)_u$-, $HS(CH_2)C(O)$-$(CH_2)_u$-, $H_2N(CH_2)_4C(O)$-$(CH_2)_u$- and $HOCH_2C(O)$-$(CH_2)_u$- wherein u is 0 or an integer from 1 to 10. The preferred non-peptidic

groups enhance the stability, bioavailability or activity of the peptides. Suitable non-peptidic groups include, but are not limited to hydrophobic groups such as carbobenzoxyl, dansyl, t-butyloxycarbonyl, acetyl, 9-fluorenylmethoxycarbonyl, groups which stabilize or mimic alpha-helices, groups which mimic the secondary structure of peptides, particularly alpha helical peptides, such as those disclosed in WO 03/018587, groups which improve bioavailability, such as hydrophilic groups which aid aqueous solubility, for example, cyclodextrans; groups which are recognised by transport receptors to allow or improve transport of the peptides to the site of activity, for example, transport across cell walls or through an epithelial layer such as skin or the gut wall;

R_2 is selected from H, a C-terminal capping group that stabilizes the terminus of a helix, usually having hydrogen atoms able to form hydrogen bonds or having a positive charge at the C-terminus to match with the helix dipole, a peptide of 1 to 15, 1 to 10 or 1 to 5 amino acid residues optionally capped with a C-terminal capping group that stabilizes the terminus of a helix, usually having hydrogen atoms able to form hydrogen bonds or having a positive charge at the C-terminus to match with the helix dipole, a mimic of an amino acid side chain or a group which activates the terminal carboxylic acid carbonyl group to nucleophilic substitution. A suitable C-terminal capping group is NH_2. Suitable mimics of amino acid side chains are any common or unnatural amino acid side chain attached to an amine group which forms an amide bond with the C-terminal carboxylic acid. Suitable mimics of amino acid side chains include but are not limited to:

$-(CH_2)_u-NHCH_2CH_3$, $-(CH_2)_u-NH(CH_2)_4NHC(=NH)NH_2$, $-(CH_2)_u-NH(CH_2)_2C(O)-NH_2$, $-(CH_2)_u-NH-(CH_2)_2CO_2H$, $-(CH_2)_u-NH-(CH_2)_2-SH$, $-(CH_2)_u-NH(CH_2)_3C(O)NH_2$, $-(CH_2)_u-NH(CH_2)_3-CO_2H$, $-(CH_2)_u-NH-(CH_2)_2$(4-imidazolyl), $-(CH_2)_u-NH-CH_2CH(CH_3)CH_2CH_3$, $-(CH_2)_u-NH-(CH_2)_2CH(CH_3)_2$, $-(CH_2)_u-NH-(CH_2)_5NH_2$, $-(CH_2)_u-NH-(CH_3)_3SCH_3$, $-(CH_2)_u-NH-(CH_2)_2$(3-indolyl), $-(CH_2)_u-NH-(CH_2)_2$(4-hydroxyphenyl), $-(CH_2)_u-NH(CH_2)_3$(4-hydroxyphenyl), $-(CH_2)_u-NH-CH_2CH(CH_3)_2$, $-(CH_2)_u-NHCH_2CH_2CH_3$, $-(CH_2)_u-NH-CH_2C_6H_{10}$, $-(CH_2)_u-NH-CH_2C_5H_8$, $-(CH_2)_u-NHCH_3$, $-(CH_2)_u-NH(CH_2)_4CH_3$, $-(CH_2)_u-NH(CH_2)_5CH_3$, $-(CH_2)_u-NHCH_2CO_2H$, $-(CH_2)_u-NHCH_2-SH$, $-(CH_2)_u-NH-(CH_2)_2OH$, $-(CH_2)_u-NH-(CH_2)_5NH_2$ and $-(CH_2)_u-NH-CH_2OH$; wherein u is 0 or an integer from 1 to 10.

Suitable groups which activate the C-terminal carboxylic to nucleophilic attack include converting the carboxylic acid to an acid chloride, an acid anhydride, an acyl azide, an O-acylisourea, a phosphonium derivative or an activated ester, especially those known in the art for activating carboxylic acids for peptide bond formation;

The preferred non-peptidic groups enhance the stability, bioavailability or activity of the peptides. Suitable non-peptidic groups include but are not limited to hydrophobic groups such as t-butyl, groups which stabilize or mimic alpha-helices, groups which mimic the secondary structure of peptides, particularly alpha helical peptides, such as those disclosed in WO 03/018587, groups which improve bioavailability, such as hydrophilic groups which aid aqueous solubility, for example, cyclodextrans; groups which are recognised by transport receptors to allow or improve transport of the peptides to the site of activity, for example, transport across cell walls or through an epithelial layer such as skin or the gut wall;

Each R′ is selected from H, CH_3, CH_2CH_3, vinyl, OH, OCH_3, NH_2, $NH(CH_3)$, $N(CH_3)_2$, phenyl, F or Cl; most preferably H or CH_3, especially H;

Each R″ is selected from H, CH_3, CH_2CH_3 or vinyl, especially H;

m is 1 and n is 3 or 4, m is 2 and n is 4, m is 3 and n is 1 or m is 4 and n is 1 or 2, especially where m is 1 and n is 4;

Each Xaa may be any amino acid residue selected to mimic the amino acid residues in a known alpha helical peptide of interest or to prepare an unknown peptide having new properties. Xaa is preferably a D- or L- alpha amino acid residue. Especially preferred peptides of formula (I) have at least one Xaa which is a D- or L- alpha amino acid residue that is favourable to helix formation. Even more preferred are peptides in which 2 or 3 of Xaa are D- or L- alpha amino acid residues that are favourable to helix formation, for example, alanine, arginine, lysine, methionine, leucine, glutamic acid, glutamine, cysteine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine and aspartic acid, especially alanine, arginine, lysine, methionine, leucine, glutamic acid and glutamine;

L is –NH-C(O)- or –C(O)-NH-;

Preferably p is selected to provide the appropriate number of turns in the alpha helix. Especially preferred are those peptides where p is 2 to 11, 2 to 10, 2 to 9 or 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4, 2 to 3, 3 to 10, 3 to 9, 3 to 8, 3 to 7, 3 to 6, 3 to 5, especially 2 to 5.

[0093] Preferred peptides containing more than one consecutive macrocyclic module include those of formula (V):

$$R_1\text{-}[1,5\text{-cyclo(Zaa-XaaXaaXaa-Yaa)}]_q\text{-}R_2 \qquad (V)$$

wherein each 1,5-cyclo(Zaa-XaaXaaXaa-Yaa) is independently selected from:

cyclo-1,5-KXaaXaaXaaD	SEQ ID NO: 18
cyclo-1,5-DXaaXaaXaaK	SEQ ID NO: 19
cyclo-1,5-KXaaXaaXaaE	SEQ ID NO: 20
cyclo-1,5-EXaaXaaXaaK	SEQ ID NO: 21
cyclo-1,5-OXaaXaaXaaD	SEQ ID NO: 22 and
cyclo-1,5-DXaaXaaXaaO	SEQ ID NO: 23

q is an integer from 2 to 12 and R_1 and R_2 are as defined above.

[0094] Illustrative examples of 1,5-cyclo(ZaaXaaXaaXaa-Yaa) sequences include:

cyclo-1,5-KARAD	SEQ ID NO: 24
cyclo-1,5-DARAK	SEQ ID NO: 25
cyclo-1,5-KARAE	SEQ ID NO: 26
cyclo-1,5-EARAK	SEQ ID NO: 27
cyclo-1,5-OARAD	SEQ ID NO: 28
cyclo-1,5-DARAO	SEQ ID NO: 29
cyclo-1,5-KAAAD	SEQ ID NO: 30 and
cyclo-1,5-KGSAD	SEQ ID NO: 31.

[0095] In another embodiment, individual macrocyclic modules in the peptide are different.

[0096] In yet another embodiment, individual macrocyclic modules in the peptide are the same.

[0097] Examples of peptides containing more than one consecutive cyclic pentapeptide module which are very stable alpha helices in water include:

cyclo(1-5, 6-10)-Ac-[KARADKARAD]-NH_2 SEQ ID NO: 32 and

cyclo(1-5, 6-10, 11-15)-Ac-[KARADKARADKARAD]-NH_2 SEQ ID NO: 33.

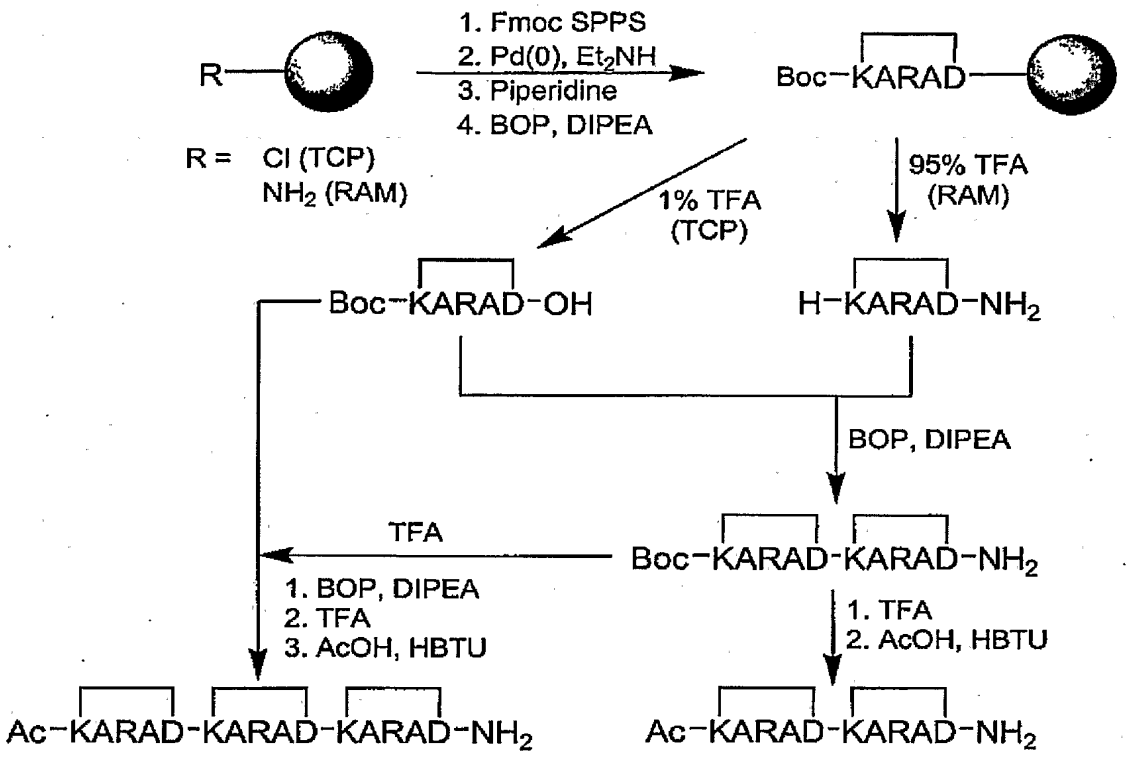
[0098] Peptides comprising more than one macrocyclic module can be prepared by conventional solid phase synthesis as described for single macrocycles above, where cyclization occurs while the peptide is still attached to the solid phase resin by

incorporation of amino acid residues with suitably protected side chains such as allyl protected aspartic acid or alloc protected lysine, deprotection and cyclization. Further amino acid residues may be added to the resin bound macrocycle including other amino acid residues with suitable protected side chains, after the addition of five further amino acids, further cyclization may be effected to provide two consecutively linked macrocycles. This may be continued until the desired number of macrocycles is present and then the peptide can be cleaved from the resin.

[0099] Alternatively, a single cyclic macrocyclic module may be prepared using solid phase synthesis as hereinbefore described. The single macrocyclic module may be cleaved from the resin and undergo either N-terminal protection or deprotection or C-terminal protection or deprotection. A macrocycle having N-terminal protection and a macrocycle having C-terminal protection may then be reacted with one another by activating the unprotected carboxylic acid to nucleophilic attack by the unprotected amine nitrogen, to provide a multi-macrocyclic structure. Further N-terminal and/or C-terminal protection and deprotection of a single macrocyclic module and a multi-macrocyclic module followed by coupling will allow the preparation of a multi-macrocyclic peptide.

[0100] Two macrocyclic modules may be coupled using conventional peptide coupling chemistry. For example, the C-terminal carboxylic acid may be activated by formation of an acid chloride, acid anhydride, an acyl azide, a carbodiimide, an acyloxyphosphonium compound or an active ester, and allowing nucleophilic attack from the N-terminal nitrogen atom. A particularly preferred method of activating the carboxylic acid to nucleophilic attack is preparation of an acyloxyphosphonium derivative of the carboxylic acid, for example, by reaction with the carboxylic acid with BOP, Py-BOP or HBTU in the presence of a tertiary amine such as triethylamine or diisopropylethylamine.

[0101] A representative synthesis of a multi-macrocyclic peptide where each macrocyclic module is the same is shown in Scheme 2.

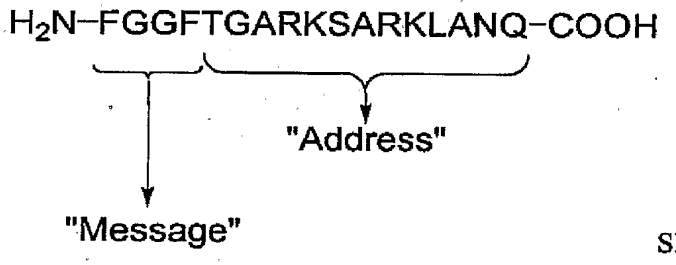


Scheme 2

[0102] The helically constrained peptides described herein can be synthesised with additional chemical groups present at their amino and/or carboxy termini, such that, for example, the stability, bioavailability, and/or inhibitory activity of the peptides is enhanced. For example, hydrophobic groups such as carbobenzoxyl, dansyl, or t-butyloxycarbonyl groups, may be added to the amino termini. An acetyl group or a 9-fluorenylmethoxy-carbonyl group may be placed at the amino termini. A hydrophobic group, t-butyloxycarbonyl, or an amido group may be added to carboxy termini. Furthermore, the peptides of the invention can be synthesised such that their steric configuration is altered. For example, the D-isomer of one or more of the amino acid residues of the peptide can be used, rather than the usual L-isomer. The compounds can contain at least one bond linking adjacent amino acids that is a non-peptide bond, and is preferably not helix breaking. Non-peptide bonds for use in flanking sequences include an imino, ester, hydrazine, semicarbazide, oxime, or azo bond. Still further, at least one of the amino acid residues of the peptides of the invention can be substituted by one of the well known non-naturally occurring amino acid residues, that is preferably not helix breaking. Desirably, the non-natural amino acid or non-amide bond linking adjacent amino acids,

when present, is present outside of the internal sequence, and is, preferably, not helix breaking. Still further, at least one of the amino acid residues of the peptides of the invention can be substituted by one of the well known non-naturally occurring amino acid residues. Alterations such as these can serve to increase the stability, bioavailability, immunogenicity, and/or inhibitory action of the peptides of the invention.

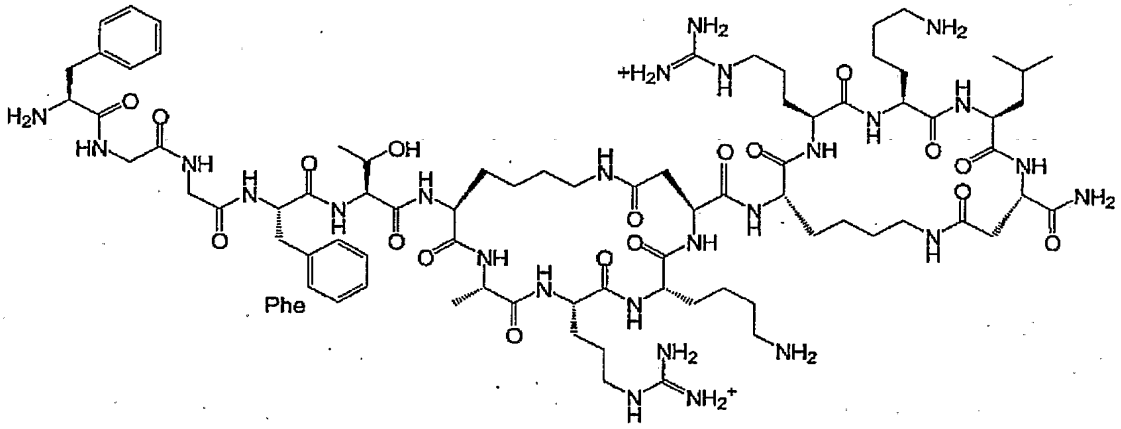
[0103] A representative example of alpha helical cyclic pentapeptides incorporated in a modular fashion into biologically active sequences is described. The opioid receptor-like 1 (ORL-1) is the most recently identified member of the opioid receptor family [96]. Unlike the other three types of opioid receptor (μ, δ, κ), the ORL-1 receptor does not display affinity for the naturally occurring opioid peptide ligands or for many synthetic opiates that selectively bind μ–, δ–, κ–receptors [97]. In 1995 the endogenous ligand for the ORL-1 receptor was identified and called nociceptin (NC). Like other opioid receptor peptide ligands nociceptin consists of an N-terminal tetrapeptide which is referred to as the "message" sequence and is primarily responsible for triggering stimulation of the receptor, whilst the remaining C-terminal portion is referred to as the "address" sequence and is involved in binding and receptor specificity[96].



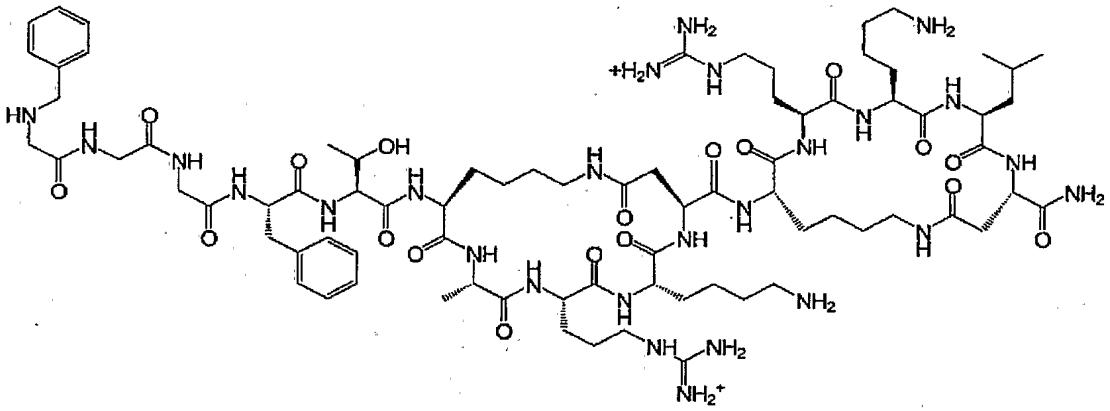
SEQ ID NO: 34

[0104] Recent NMR structures of NC and related peptides revealed a highly helical structure in the address domain and suggested amphipathicity[98-100]. Another recent report successfully substituted Aib residues into the NC address sequence resulting in increased potency and affinity in 13-residue peptide sequences. Structure-activity relationship (SAR) studies suggest the minimal sequence is NC 1-13. An alanine scan showed the first five residues (FGGFT) are critical, whilst G6 and A7 appear to tolerate substitution, R8 is highly crucial, whilst the remaining residues are necessary but tolerate alanine substitution [101,102]. Another recent report identified a pure, selective peptide antagonist of the ORL-1 receptor which involved replacing the first residue in the native sequence with Nphe[97].

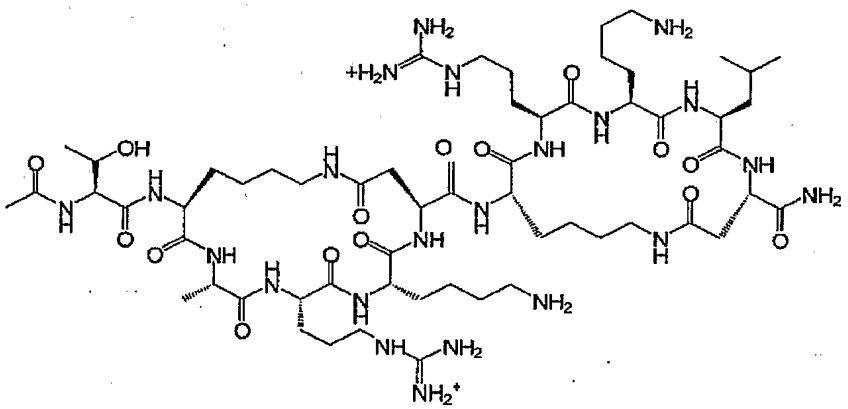
[0105] Since the present invention establishes a general method for constraining short peptides into α-helical conformations, nociceptin is an ideal target to show that constraining biologically important helices into an α-helical conformation can improve activity and affinity. Thus the peptides of SEQ ID NOs: 37 to 39 were designed using the available SAR. The peptide of SEQ ID NO: 37 is designed to be a nociceptin mimetic for agonism, whilst the peptide of SEQ ID NO: 38 is based on the recently reported antagonist [Nphe1]NC (1-15) . The peptide of SEQ ID NO: 39 consists of just the address sequence and we envisage that if this peptide has a high enough affinity for the receptor it may function as an antagonist, there are no studies to date on peptides incorporating only the address sequence.



FGFT[1,4-cyclo(KARKD)][1,4-cyclo(KRKVD)]-NH_2 SEQ ID NO: 35



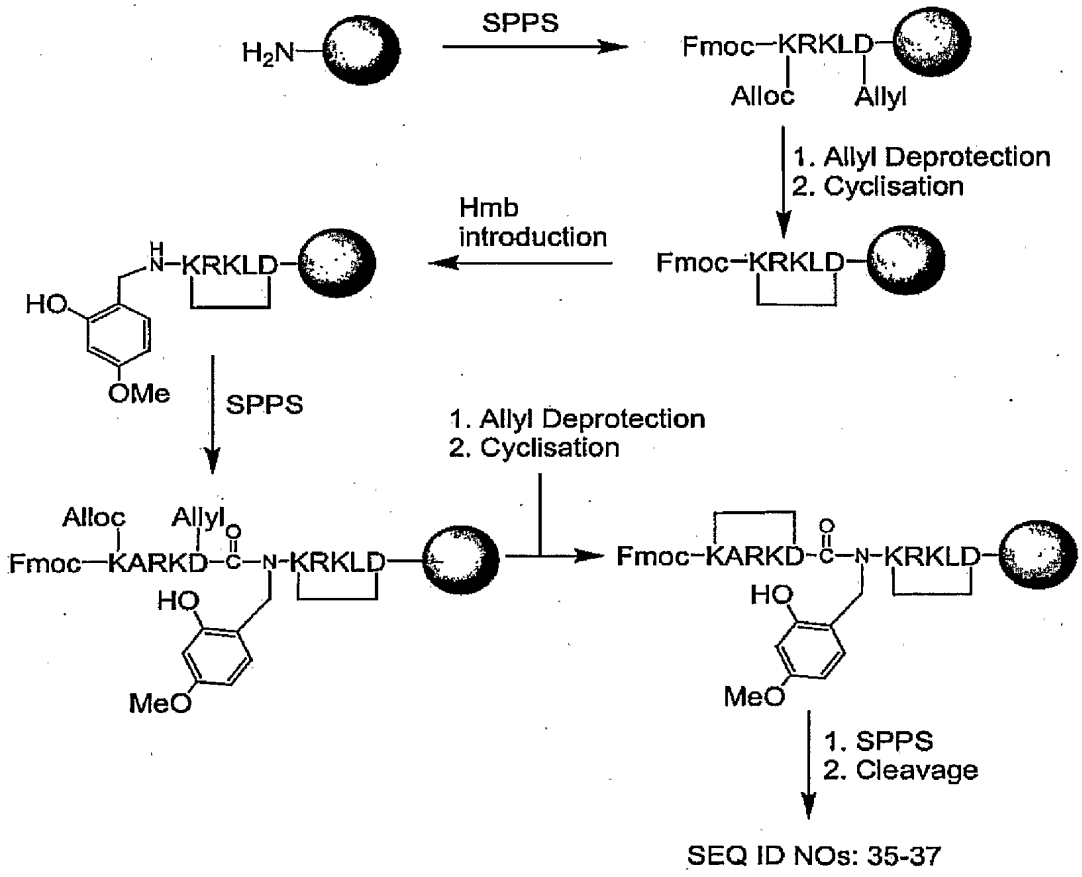
$PhCH_2$-GGGFT[1,4-cyclo(KARKD)][1,4-cyclo(KRKVD)]-NH_2 SEQ ID NO: 36



Ac-T-[1,4-cyclo(KARKD)][1,4-cyclo(KRKVD)]-NH_2 SEQ ID NO: 37

[0106] CD spectra of the nociceptin mimetics SEQ ID NO: 35 and SEQ ID NO: 37 reveal their highly helical nature compared to the known linear agonist FGGFTGARKSARK-NH_2 (SEQ ID NO: 38), antagonist NPheGGFTGARKSARK-NH_2 (SEQ ID NO: 39), and address sequence Ac-TGARKSARK-NH_2 (SEQ ID NO: 40). Based on these results the present inventors envisage constraining this region into a helical conformation should increase the affinity of SEQ ID Nos: 35 to 37 for the ORL-1 receptor.

[0107] A representative synthesis of the Nociceptin mimetics of SEQ ID NOs: 35 to 37 is shown in Scheme 3. The 2-hydroxy-4-methoxybenzyl protecting group is used during synthesis of multi-macrocyclic compounds, however this group is removed during deprotection and cleavage of the peptides from the resin.



Scheme 3

[0108] The present invention also provides compositions which comprise one or more compounds of the invention. The compounds themselves may be present in the

compositions in any of a wide variety of forms. For example, two or more compounds may be merely mixed together or may be more closely associated through complexation, crystallization, or ionic or covalent bonding.

[0109] Those of skill in the art will appreciate that a wide variety of prophylactic, diagnostic, and therapeutic treatments may be prepared from the compounds and compositions of the present invention, due in large part to the cross-reactivity – i.e., agonism or antagonism – of the macrocyclic moieties of the compounds with one or more naturally-occurring peptides or polypeptides. Thus, a compound of the present invention finds utility as a molecular mimic or antagonist of a member of a ligand-receptor binding pair that underlies or is otherwise associated with the development of a particular disease or condition, wherein the ligand-receptor interaction is mediated at least in part by one or more alpha helical motifs present in the ligand or the receptor. Accordingly, in some embodiments, a compound of the present invention having one or more macrocyclic moieties that antagonize the interaction of a ligand and a receptor will be useful in the prevention or treatment of a disease or condition that results from inappropriate activation of the receptor by the ligand. In other embodiments, a disease or condition may arise through inadequate activation of a receptor, in which case the disease or condition may be treated or prevented by means of a compound having one or more macrocyclic moieties that mimic the binding determinants of the ligand or the receptor. Illustrative diseases or conditions mediated by alpha-helix associated ligand-receptor interactions include diseases or conditions related to DNA transcription, diseases related to RNA reverse transcription, diseases or disorders related to transcriptional antitermination, diseases related to apoptosis regulation and tumor suppression, for example, cancers such as brain tumors, breast cancer, lung cancer, bone cancer, colon cancer, ovarian cancer, testicular cancer, renal cancer, liver cancer, lymphoma and leukemia; diseases or disorders related to calcium homeostasis, diseases or disorders related to pain transmission, diseases or disorders associated with lipid metabolism and cholesterol homeostasis, diseases and disorders related to stress response

[0110] Thus, a further aspect of the invention contemplates a method for treating or preventing a disease or condition associated with a ligand-receptor interaction that is mediated at least in part by an alpha helical domain present in the ligand or the receptor, comprising administering an effective amount of a compound comprising at least one alpha helical cyclic peptide, wherein each peptide comprises a sequence of five amino acid

residues having a first terminal residue and a second terminal residue that are separated by an intervening sequence of three amino acid residues, and wherein the side chains of the first and second terminal residues are linked to each other and wherein the side chains of at least some of the amino acid residues of the or each peptide are in a (three-dimensional) configuration that is analogous to the configuration of amino acid side chains of at least a portion of the alpha helical domain of the ligand or the receptor. Preferably the compound is a compound of any one of formula (I), (II) or (IV).

[0111] As used herein, the term "effective amount" relates to an amount of compound which, when administered according to a desired dosing regimen, provides the desired mediation of the disease or disorder, therapeutic activity or disease prevention. Dosing may occur at intervals of minutes, hours, days, weeks, months or years or continuously over any one of these periods. A therapeutic, or treatment effective amount is an amount of the compound which, when administered according to a desired dosing regimen, is sufficient to at least partially attain the desired therapeutic effect, or delay the onset of, or inhibit the progression of or halt or partially or fully reverse the onset or progression of the disease or disorder. A prevention effective amount of compound which when administered to the desired dosing regimen is sufficient to at least partially prevent or delay the onset of a particular disease or condition.

[0112] Yet another aspect of the invention provides a use of a compound comprising an alpha helical cyclic peptide, wherein the peptide comprises a sequence of five amino acid residues having a first terminal residue and a second terminal residue that are separated by an intervening sequence of three amino acid residues, and wherein the side chains of the first and second terminal residues are linked to each other, in the preparation of a medicament for the treatment or prevention of a disease or disorder mediated by the interaction of alpha helical peptides with biomolecules.

[0113] Suitable dosages may lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage. The dosage is preferably in the range of 1 µg to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage is in the range of 1 mg to 500 mg per kg of body weight per dosage. In another embodiment, the dosage is in the range of 1 mg to 250 mg per kg of body weight per dosage. In yet another preferred embodiment, the dosage is in the range of 1 mg to 100 mg per kg of body weight per dosage, such as up to

50 mg per kg of body weight per dosage. In yet another embodiment, the dosage is in the range of 1μg to 1mg per kg of body weight per dosage.

[0114] Suitable dosage amounts and dosing regimens can be determined by the attending physician and may depend on the severity of the condition as well as the general age, health and weight of the subject.

[0115] The active ingredient may be administered in a single dose or a series of doses. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a composition, preferably as a pharmaceutical composition.

[0116] According to a further aspect, the invention contemplates a pharmaceutical composition comprising a compound comprising an alpha helical cyclic peptide, wherein the peptide comprises a sequence of five amino acid residues having a first terminal residue and a second terminal residue that are separated by an intervening sequence of three amino acid residues, and wherein the side chains of the first and second terminal residues are linked to each other, a conformationally constrained peptide having a plurality of alpha helical pentapeptide sequences, wherein the pentapeptide sequences comprise a sequence of five amino acid residues having a first terminal residue and a second terminal residue that are separated by an intervening sequence of three amino acid residues, and wherein the side-chains of the first and second terminal residues are linked to each other, or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier, excipient or diluent.

[0117] Suitable pharmaceutically acceptable salts include, but are not limited to, salts of pharmaceutically acceptable inorganic acids such as hydrochloric, sulphuric, phosphoric, nitric, carbonic, boric, sulfamic, and hydrobromic acids, or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, malic, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, toluenesulphonic, benzenesulphonic, salicylic, sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

[0118] Base salts include, but are not limited to, those formed with pharmaceutically acceptable cations, such as sodium, potassium, lithium, calcium, magnesium, zinc, ammonium, alkylammonium such as salts formed from triethylamine, alkoxyammonium such as those formed with ethanolamine and salts formed from ethylenediamine, choline or amino acids such as arginine, lysine or histidine.

[0119] Basic nitrogen-containing groups may be quarternised with such agents as lower alkyl halide, such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates like dimethyl and diethyl sulfate; and others.

[0120] The formulation of such compositions is well known to those skilled in the art. The composition may contain pharmaceutically acceptable additives such as carriers, diluents or excipients. These include, where appropriate, all conventional solvents, dispersion agents, fillers, solid carriers, coating agents, antifungal and antibacterial agents, dermal penetration agents, surfactants, isotonic and absorption agents and the like. It will be understood that the compositions of the invention may also include other supplementary physiologically active agents.

[0121] The carrier must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the composition and not injurious to the subject. Compositions include those suitable for oral, rectal, inhalational, nasal, transdermal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intraspinal, intravenous and intradermal) administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

[0122] Depending on the disease or condition to be treated, it may or may not be desirable for a compound of Formula (I) or (IV) to cross the blood/brain barrier. Thus the compositions for use in the present invention may be formulated to be water or lipid soluble.

[0123] Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

[0124] A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a

suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (eg inert diluent, preservative, disintegrant (eg. sodium starch glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose)) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

[0125] Compositions suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured base, usually sucrose and acacia or tragacanth gum; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia gum; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

[0126] The compounds of Formula (I) or (IV) may also be administered intranasally or via inhalation, for example by atomiser, aerosol or nebulizer means.

[0127] Compositions suitable for topical administration to the skin may comprise the compounds dissolved or suspended in any suitable carrier or base and may be in the form of lotions, gel, creams, pastes, ointments and the like. Suitable carriers include mineral oil, propylene glycol, polyoxyethylene, polyoxypropylene, emulsifying wax, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. Transdermal devices, such as patches, may also be used to administer the compounds of the invention.

[0128] Compositions for rectal administration may be presented as a suppository with a suitable carrier base comprising, for example, cocoa butter, gelatin, glycerin or polyethylene glycol.

[0129] Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

[0130] Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers,

bactericides and solutes which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

[0131] Preferred unit dosage compositions are those containing a daily dose or unit, daily sub-dose, as herein above described, or an appropriate fraction thereof, of the active ingredient.

[0132] It should be understood that in addition to the active ingredients particularly mentioned above, the compositions of this invention may include other agents conventional in the art having regard to the type of composition in question, for example, those suitable for oral administration may include such further agents as binders, sweeteners, thickeners, flavouring agents, disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

BRIEF DESCRIPTION OF THE FIGURES

[0133] Figure 1 depicts a CD spectrum of SEQ ID NOs: 8 to 13 and 38 and 39.

[0134] Figure 2 depicts a CD spectrum of SEQ ID NOs: 14 to 17.

[0135] Figure 3 depicts a CD spectrum comparing the helicity of SEQ ID NOs: 32 and 33 with their acyclic linear analogues SEQ ID NOs: 43 and 44.

[0136] Figure 4 depicts the Sequential and medium ROEs, temperature coefficients, and coupling constants for SEQ ID NO: 32 in 90% H_20: 10% D_2O.

[0137] Figure 5 depicts (a) Helical wheel for dimer SEQ ID NO: 32, cyclo(1-5,6-10)-Ac-[KARADKARAD]-NH_2 showing side chain distribution; (b) side view of SEQ ID NO: 32 with helical backbone (yellow), bridging lactam restraints (white), exposed side chains (green spheres); and (c) SEQ ID NO: 32 viewed end on.

[0138] Figure 6 depicts CD spectra in 10 mM phosphate buffer, pH 7.4, 25°C for 32-44 mM solutions of (a) SEQ ID NO: 32 (–), SEQ ID NO: 33 (– - - –) and acyclic analogues SEQ ID NO: 48 (– –) and SEQ ID NO: 45 (- - -); (b) SEQ ID NO: 32 (–) versus SEQ ID NO: 32 (- - -), SEQ ID NO: 44 (– –) and SEQ ID NO: 45 (– - –) in 50% TFE.

[0139] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

[0140] The invention will now be described with reference to the following examples which are included for the purpose of illustration only and are not intended to limit the generality of the invention hereinbefore described.

EXAMPLES

GENERAL

[0141] Fmoc-Asp(OAllyl)-OH and tetrakis(triphenylphosphino)palladium were obtained from Sigma-Aldrich (Sydney, Australia). Boc-Lys(Fmoc)-OH, Rink Amide MBHA resin and other L-amino acids were obtained from Novabiochem (Melbourne, Australia). Benzotriazol-1-yl-1,1,3,3-tetramethyluronium (HBTU) and benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium (BOP) were obtained from Richelieu Biotechnologies (Quebec Canada). All other reagents were of peptide synthesis grade and obtained from Auspep (Melbourne, Australia).

NMR SPECTROSCOPY.

[0142] Samples for NMR analysis of peptides were prepared by dissolving the peptide 3mg in 450ul H_2O and 50ul D_2O (5mmol) and adjusting the pH of the solution to 4.5 by adding HCl or NaOH and stirring for 30 mins. 1D and 2D ^1H NMR spectra were recorded on both Bruker ARX-500 and Bruker Avance DMX-750 spectrometers at 278K. All spectra were recorded in the phase sensitive mode using time proportional phasing incrementation[96]. 2D experiments included TOCSY using MLEV-17 spin lock sequence with a mixing time of 100ms, NOESY with a mixing time of 300ms. Water suppression was achieved using watergate W5 pulse sequences with gradients using double echo[97]. 2D TOCSY and NOESY experiments were recorded over 7936.5 Hz with 4096 complex data points in F2 and 512 increments in F1 with 16 and 48 scans per increment respectively. Spectra were processed using XWINNMR (Bruker, Germany). The $t1$ dimensions of all 2D spectra were zero filled with 2048 real data points, and 90° phase-shifted sine bell window functions applied in both dimensions followed by fourier transformation and fifth order polynomial baseline correction. Chemical shifts were referenced to TSP an internal standard at 0.00ppm. Processed spectra were analyzed using the program SparkyNMR[98] and assigned using the sequential assignment technique[99].

STRUCTURE CALCULATIONS.

[0143] Cross peaks in NOESY spectra were integrated and calibrated in SparkyNMR[98], and distance constraints derived using the standard CALIBA function in DYANA[100]. Corrections for pseudo atoms were added to distance constraints where needed. Backbone dihedral angle restraints were inferred from $^3J_{NHCH\alpha}$ coupling constants in 1D spectra at 278K and 288K, ϕ was restrained to $-65 \pm 30°$ for $^3J_{NHCH\alpha} \leq 6$Hz. Peptide

bond ω angles were all set to trans, and structures were calculation without explicit hydrogen bond restraints. Stereospecific assignments of β-methylene protons and χ_1 dihedral angles were derived from 1D ^{1}H spectra $^3J_{\alpha\beta}$ and set to –60 ± 30° for both aspartic acid residues. Initial structures were generated using a torsion angle simulated annealing protocol in DYANA until no violations were obtained. Final structures were calculated using XPLOR 3.851. Starting structures with randomised ϕ and ψ angles and extended side chains were generated using an ab initio simulated annealing protocol[101]. The calculations were performed using the standard forcefield parameter set (PARALLHDG.PRO) and topology file (TOPALLHDG.PRO) in XPLOR with in house modifications to generated lactam bridges between lysing and aspartic acid residues. Refinement of structures was achieved using the conjugate gradient Powell alogrithm with 1000 cycles of energy minimsation and a refined forcefield based on the program CHARMm[102]. Structures were visualised with MOLMOL[103] and InsightII[104].

CD SPECTROSCOPY.

[0144] CD experiments were performed on a Jasco Model J-710 spectropolarimeter which was routinely calibrated with (1S)-(+)-10-camphorsulfonic acid. Temperature control was achieved using a Neslab RTE-111 circulating water bath. Spectra were recorded in a 0.1cm Jasco cell between 310-185nm at 50 nm/min wit a band width of 1.0nm, response time of 2 sec, resolution step width of 0.1nm and sensitivity of 20, 50 or 100 mdeg. Each spectrum represents the average of 5 scans with smoothing to reduce noise. Peptide samples for CD spectroscopy were dissolved in distilled water (~1mg/ml). Each stock solution was diluted to a final concentration of 50uM in 10mM sodium phosphate buffer (pH7.4), with or without additives (2,2,2-trifluoroethanol (TFE) or guanidine.HCl). Guanidine.HCl denaturation experiments were performed according to Creighton[105].

[0145] Accurate concentration determination of stock solutions were obtained by 1D ^{1}H NMR using the method of Larive *et. al.*[106] (425µl of peptide stock solution, 50µl of D_2O, and 25µl of 10.077mM DSS as an internal standard).

EXAMPLE 1

[0146] Synthesis of peptides SEQ ID NO. 8 to SEQ ID NO: 17 was achieved via standard Fmoc solid phase peptide synthesis on Rink amide MBHA resin. Orthogonal protection utilised allyl and alloc protection on aspartic/glutamic acids and lysine/ornithine

residue side chains respectively. Once the five or six residue peptides had been assembled and the N-terminus acetylated, the orthogonally protected side chains were unmasked utilising N.N-dimethylbarbituric acid (3eq) and acetic acid (1.5 eq) in combination with palladium (tetrakis)triphenylphosphine (5-10 mol%). Once deprotection was effected cyclisation of the side chain residues was achieved on resin with benzotriazolyloxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP) (1.2eq) and diisopropylethylamine (DIPEA) (2eq). Final cleavage and deprotection was effected with TFA/TIPS.

EXAMPLE 2

[0147] Cyclic peptides cyclo-1,4-Ac[OARAE]-NH_2 SEQ ID NOs: 38 and cyclo-1,4-Ac[EARAO]-NH_2 SEQ ID NO: 39 were prepared in an analogous manner to the peptides in Example 1.

EXAMPLE 3

[0148] Synthesis of the peptide of formula (II) was achieved by standard Fmoc SPPS protocols using trityl chloride polystyrene resin. The peptide was capped with phenyl butanoic acid, cleaved from the resin using 1% TFA in dichloromethane (DCM) leaving side chain protecting groups intact. Isobutylamine was then coupled on using BOP, DIPEA, with $CuCl_2$ – an additive known to minimize racemisation of the C-terminal residue. Following this final deprotection was effected with 95% TFA, 2.5% TIPS, 2.5% H_2O.

EXAMPLE 4

[0149] *NH_2-(cyclo1-5)-KARAD-NH_2* (SEQ ID NO: 40) was prepared by manual stepwise solid phase peptide synthesis using HBTU/DIPEA activation for Fmoc chemistry[107] on Rink Amide MBHA resin (substitution 0.78mmol.g^{-1}, 1.56mmol, 2000mg). Four equivalents of amino acid and eight equivalents of diisopropylethylamine (DIPEA) were employed in each coupling step (45mins), except for Fmoc-Asp(OAllyl)-OH and Boc-Lys(Fmoc)-OH where only 2 equivalents were used. Fmoc deprotections were achieved with 3×5min treatments with excess 1:1 piperidine:DMF. Coupling yields were monitored by quantitative ninhydrin assay[108] and double couplings were employed for yields below 99.6%. After the assembly was complete, the allyl ester of aspartic acid was removed by treating the peptide resin with $Pd(PPh_3)_4$ (0.05eq) and diethylamine (5eq) in DCM, under argon and in the dark for 2hrs. After which the peptide was washed with

DCM, DMF and 0.5% diethyldithiocarbamate in DMF. 2mg of resin was subjected to cleavage and the progress of the reaction monitored by Mass spectrometry (MS). This process was repeated if necessary. Following Allyl ester deprotection the N(ζ)-Fmoc group was removed by treatment with piperidine (1:1 in DMF). Cyclisation was effected on-resin using 1.5 eq BOP, 2eq DIPEA in DMF/Benzene (2:1). The reaction was monitored by cleaving ~2mg resin and subjecting the residue to MS, total reaction time was approximately 48-72 hours. The peptides were simultaneously deprotected and cleaved from the resin by 2hr treatment of the washed and dried resin in 95% TFA, 2.5% TIPS, 2.5% H_2O (15µl per 10mg resin). The solution was then filtered, the filtrate concentrated *in vacuo* and the peptide precipitated with cold diethyl ether. The peptide precipitate was filtered washed with copious amounts of diethyl ether, redissolved in 1:1 acetonitrile/water and lyophilised. The crude peptides were purified by rp-HPLC (Vydac C18 column, 300Å. 22 × 250mm, 214nm, Solvent A = 0.1% TFA in H_2O, Solvent B = 0.1% TFA, 10% H_2O in Acetonitrile. Gradient: 0%B to 100%B over 30 mins. Yield 30% (isolated). [R_t=12.82min]. MS: [M+H^+] (calc.) = calc. 541.31 (expt.)=541.39 .

EXAMPLE 5

[0150] *Boc-(cyclo1-5)-KAR(Pbf)AD-OH* (SEQ ID NO: 41) was synthesised in an analogous manner to peptide (SEQ ID NO: 40), however using trityl chloride resin (0.95mmol.g^{-1}, 1.28g, 1.16mmol). Cleavage was achieved using 50ml 10% acetic acid, 20% 2,2,2-trifluoroethanol, 70% DCM for 2hrs. After lyophilisation the crude peptide was deemed pure enough by analytical HPLC and used without further purification. Yield 50%. MS: [M+H^+] (calc) = 893.43 (expt.) = 893.67]

EXAMPLE 6

[0151] DIPEA (135µL, 0.38 mmol) was added to a solution of SEQ ID NO: 41 (154mg, 0.17mmol), SEQ ID NO: 40 (102mg, 0.19mmol, and BOP (80mg, 0.18mmol) in DMF (5mL). After stirring (2h, RT), solvent was evaporated in vacuo, the residue dissolved in H_2O/MeCN (1:1), lyophilised and purified (rpHPLC). The product was treated with TFA/TIPS 19:1 (1h, 20°C), evaporated, and reacted (2h, 20°C) with AcOH (15ul, 0.26mmol), 0.5M HBTU (500 µL 0.25mmol) and DIPEA (90uL, 0.52mmol). Solvent was removed in vacuo, H_2O/MeCN (1:1) added, lyophilised and purified (rpHPLC) to yield SEQ ID NO: 32 (19.1mg, 10% isolated). MS [M+H^+] (calc.) 1106.6 (expt.) 1106.97,

[M+2H]/2 (calc.) = 554.3 (expt.) = 554.04. Anal. rpHPLC: 14.8 min. (Gradient 0%-100% acetonitrile over 30 min).

EXAMPLE 7

[0152] DIPEA (135µL, 0.38 mmol) was added to a solution Peptide SEQ ID NO: 41 (66mg, 0.077mmol), peptide SEQ ID NO: 40 (42mg, 0.074mmol, and BOP (52mg, 0.154mmol) in DMF (5mL). After stirring (2h, RT), solvent was evaporated in vacuo, the residue dissolved in H_2O/MeCN (1:1), lyophilised and purified (rpHPLC). The product (34mg, 0.024mmol) was treated with TFA/TIPS 19:1 (1h, 20°C), evaporated, and reacted (2h, RT) with peptide SEQ ID NO: 41 (20mg, 0.024mmol), BOP (15mg, 0.034mmol), and lastly DIPEA (50ul, 0.24mmol). The solvent was evaporated in vacuo, the residue dissolved in H_2O/MeCN (1:1), lyophilised and purified (rpHPLC). The product was once again treated with TFA/TIPS 19:1 (1h, 20°C), evaporated, and reacted with AcOH (2ul, 0.0132mmol), BOP 7mg, 0.016mmol) and DIPEA (19uL, 0.138mmol) for 2hrs at RT. The solvent was removed in vacuo, H_2O/MeCN (1:1) added, lyophilised and purified (rpHPLC) to yield SEQ ID NO: 33 (7.8mg, 5.5%(isolated). MS [M+2H$^+$]/2 (calc.) = 815.44 (expt.) = 815.55. [M+3H]/3 (calc.) = 543.97 (expt.) = 544.03. Anal. rpHPLC: 15.09 min.

EXAMPLE 8

[0153] Linear Peptides Ac(KARAD)$_n$-NH_2 where n=2 [SEQ ID NO: 43] and n=3 [SEQ ID NO: 44] were prepared by manual stepwise solid phase peptide synthesis using HBTU/DIPEA activation for Fmoc chemistry[107] on Rink Amide MBHA resin (substitution 0.78mmol.g^{-1}, 0.5mmol, 648mg). Four equivalents of amino acid and eight equivalents of diisopropylethylamine (DIPEA) were employed in each coupling step (45mins). Fmoc deprotections were achieved with 3×5min treatments with excess 1:1 piperidine:DMF. Coupling yields were monitored by quantitative ninhydrin assay[108] and double couplings were employed for yields below 99.6%. After assembly of the first 10 residues, the peptide resin was washed, dried and split into two portions, one portion was acetylated, whilst to the other was added the final 5 residues. N-terminal acetylation was achieved by treating the fully protected peptide with 4 equivalents of glacial acetic acid, 4 equivalents of HBTU, and 8 equivalents of DIPEA. The peptides were simultaneously deprotected and cleaved from the resin by 2hr treatment of the washed and dried resin in 95% TFA, 2.5% TIPS, 2.5% H_2O (15µl per 10mg resin). The solution was then filtered, the filtrate

concentrated *in vacuo* and the peptide precipitated with cold diethyl ether. The peptide precipitate was filtered washed with copious amounts of diethyl ether, redissolved in 1:1 acetonitrile/water and lyophilised. The crude peptides were purified by rp-HPLC (Vydac C18 column, 300Å. 22 × 250mm, 214nm, Solvent A = 0.1% TFA in H_2O, Solvent B = 0.1% TFA, 10% H_2O in Acetonitrile. Gradient: 0%B to 100%B over 30 mins. (SEQ ID NO: 45) Yield 20% (isolated). [R_t=12.65min]. MS: [M+H^+] (calc.) = 1142.63 (expt.) = 1142.75; [M+2H^+]/2 (calc.) 571.85 (expt.) =571.86. (SEQ ID NO: 46) Yield 30% (isolated) [R_t=13.16min]. MS: [M+2H^+]/2 (calc.) = 842.46 (expt.) = 842.64; [M+3H^+]/3 (calc.) 561.98 (expt.) = 562.08.

EXAMPLE 9

[0154] Synthesis of SEQ ID NOs: 35 to 37 was carried out on Rink amide MBHA resin using standard Fmoc SPPS (scheme 3). Orthogonal protection utilised allyl and alloc protection on aspartic/glutamic acids and lysine/ornthine residue side chains respectively. After assembly of the first five residues, the orthogonal protecting groups were unmasked utilising N.N-dimethylbarbituric acid (3eq) and acetic acid (1.5 eq) in combination with palladium (tetrakis)triphenylphosphine (5-10 mol%) and cyclisation was achived with BOP and DIPEA. After subsequent piperidine deprotection, the backbone amide protecting group 2-hydroxy-4-methoxybenzyl was introduced on the Nα of lysine via imine formation with 2-hydroxy-4-methoxybenzaldehyde followed by reduction with $NaBH(OAc)_3$. Use of this protecting group enables the synthesis of 2 lactam bridges to be formed on-resin without the need for solution coupling of pentapeptide cycles. Coupling of the next aspartic acid residue was achieved via the symmetrical anhydride, followed by standard Fmoc SPPS to build the remainder of the peptide. Side chain deprotection and lactam formation was effected in the same manner as before. Final cleavage of the peptides was achieved with 92.5% TFA, 2.5% TIPS, 2.5% EDT, 2.5% H_2O.

EXAMPLE 10

[0155] Circular Dichroism (CD) was performed on peptides having SEQ ID NOs: 8 to 17 and SEQ ID NOs: 38 to 39, as described above. The molar ellipticities at 222nm, 208 nm and 190 nm, ratios of ellipticities at 222nm/208nm and percentage helicity are shown in Table 6.

TABLE 6

Peptide	$[\theta]_{222}$	$[\theta]_{208}$	$[\theta]_{192}$	$\theta_{222}/\theta_{208}$	% helicity*
SEQ ID NO: 8	-15464	-17039	52191	0.98	79
SEQ ID NO: 9	-9262	-13898	16493	0.71	47
SEQ ID NO: 10	-1411	-5018	-23962	0.24	5
SEQ ID NO: 11	-7468	-14081	12260	0.57	37
SEQ ID NO: 12	-498	-1762	-8751	0.15	1
SEQ ID NO: 13	-4494	-10568	-12822	0.44	22
SEQ ID NO: 38	723	-2952	-21524	-0.32	-4
SEQ ID NO: 39	2604	-1143	-16896	-2.64	-14
SEQ ID NO: 14	-4430	-7488	2451	0.60	18
SEQ ID NO: 15	-1836	-5690	-3657	0.35	8
SEQ ID NO: 16	-16622	-21570	44791	0.82	71
SEQ ID NO: 17	-10795	-15747	10716	0.71	45

[0156] The calculated % helicities which, while probably not accurate because equations that have so far been developed to calculate alpha helicity for peptides from CD spectra have been based on long peptide sequences, reflect a rank order for helix content SEQ ID NOs: 8 > 9 > 11 > 13 > 10 > 12 > 38 > 39.

[0157] The implication of these results are that appreciable alpha helicity (50-100%) can be achieved in cyclic pentapeptides using systems like for example, SEQ ID NO: 8.

[0158] 20 membered macrocycles related to that in SEQ ID NO: 8, with the same ring size and same positioned amide linker but with different intervening amino acids between K and D at positions *i*+1, *i*+2, and *i*+3 were also examined [SEQ ID NOs: 14 to 17]. An arginine was also tacked onto the N-terminus to promote aqueous solubility but would not be expected to affect rank orders of helicity in the following compounds. The rank order for decreasing alpha-helicity in these 20-membered cyclic pentapeptides was SEQ ID NOs: 16 > 17 > 14 > 15. Peptides having SEQ ID NOs: 14 and 15 have three of the same amino acids between linking amino acids, namely Serine or Glycine, and such amino acids are known in proteins to be the least favourable to helix formation. In fact Serine is often termed a helix breaker and Glycine is often thought of as a beta/gamma turn inducer. The peptide having SEQ ID NO: 17 suggests that even with two of these amino acids present, the cyclic pentapapetide can still have appreciable alpha helicity

[0159] CD Spectra for SEQ ID NOs: 8 to 13 and 38 and 39 and SEQ ID NOs: 14 to 17 can be found in Figures 1 and 2 respectively.

EXAMPLE 11

[0160] CD was preformed on peptides having one [SEQ ID NO: 8] or more than one modular macrocycle, SEQ ID NOs: 34 and 35, as compared with corresponding linear peptides, $Ac[KARAD]_n\text{-}NH_2$ where n = 1 [SEQ ID NO: 45], n = 2 [SEQ ID NO: 43] and n = 3 [SEQ ID NO: 44]. The molar ellipticities at 222nm, 208nm and 190nm, ratios of ellipicities at 222nm/208nm and percentage helicity are shown in Table 7.

TABLE 7

Peptide	$[\theta]_{222}$	$[\theta]_{208}$	$[\theta]_{192}$	$\theta_{222}/\theta_{208}$	% helicity*
SEQ ID NO: 8	-15464	-17039	52191	0.98	79
SEQ ID NO: 32	-32340	-24957	104187	1.29	99
SEQ ID NO: 33	-31987	-23842	100811	1.34	88
SEQ ID NO: 45	-732	-5758	-14779	0.12	0.8
SEQ ID NO: 43	-1836	-8552	-12237	0.21	3
SEQ ID NO: 44	-3852	-9788	-8024	0.39	7

* % = $f_H \times 100$, $[\theta_{\infty H}]_{222}$=-44000 degcm^{2}dmol^{-1}residue^{-1} [86], k = 2.6[85].

[0161] A CD Spectrum comparing the helicity of SEQ ID NOs: 32 and 33 with their acyclic linear analogues SEQ ID NOs: 43 and 44 is shown in Figure 3.

EXAMPLE 12

[0162] To confirm this compelling CD evidence of high α-helical structure, we have also examined NMR spectra for pentapeptide SEQ ID NO:8 in 90% H_2O: 10% D_2O. We identified multiple spectral features (Fig. 6a) characteristic of α-helicity including : (i) upfield CHα chemical shifts;[109] (ii) coupling constants $^3J_{NHCH} \leq 6Hz$[110] for all amide NHs (2.2-5.2 Hz) except D_{10}; (iii) low temperature dependence of chemical shifts ($\Delta/T \leq 4$ ppb/deg) for 7 amide NHs,[111] consistent with all expected helix-defining H-bonds except for $K_1 \rightarrow D_5$; and (iv) non-sequential medium range ROEs $d_{\alpha N}(i, i+3)$, $d_{\alpha N}(i, i+4)$ and $d_{\alpha\beta}(i, i+3)$ in ROESY spectra.[100] In particular the high intensity $d_{\alpha N}(i, i+4)$ and very weak $d_{\alpha N}(i, i+2)$ ROEs are striking, indicating a lack of substantial contributions from β- or γ- turns to the conformational mix and establishing α- rather than 3_{10}- helicity.

[0163] Three dimensional structures were calculated for SEQ ID NO: 32 in water, initially using torsional angle dynamic simulated annealing in DYANA[112], followed by

dynamic simulated annealing and energy minimization in Xplor (3.851)[113] from 89 ROE (24 sequential, 38 medium range, 27 intra-residue) distance restraints, 9 phi angle restraints ($^3J_{NHCH\alpha}$, ϕ $-65 \pm 30°$) and 2 chi1 angle restraints ($^3J_{NHCH\alpha}$, χ_1 $-60 \pm 30°$). No explicit H-bond restraints were included in calculations. Final structures indicate 3 well defined α-helical turns for SEQ ID NO: 32 in water, with lactam bridges in the locations anticipated from Figure. 5.

[0164] The helical macrocycles were conformationally very stable even under protein-denaturing conditions, as illustrated by the low dependence of their CD spectra on temperature between 5-65°C (Figure 6a) and on the concentration of guanidine.HCl (Figure 6b). SEQ ID NO: 32 was also found to be highly resistant to proteolytic cleavage by trypsin (97% recovered intact after 2h), whereas the linear peptide Ac-KARADKARAD-NH_2 (SEQ ID NO: 43) was completely degraded within 30 seconds.

[0165] In summary, 10 and 15 residue peptides were engineered to form 3 and 4 consecutive α-helical turns via 2 and 3 macrocycles shown to maintain high conformational stability in water even under strong protein-denaturing conditions. CD and 2D-NMR spectra provide compelling evidence of α-helicity that could not be increased by adding TFE. Assembly of consecutive cyclic pentapeptide modules appears to be a suitable strategy for general mimicry of small α-helical protein segments that bind receptors/ligands on one helical face. Their high conformational and proteolytic stability bring enormous advantages over linear peptides, and suggest potential uses as biological probes and drug leads.

EXAMPLE 13

Trypsin Digest

[0166] Solutions of SEQ ID NO: 32 (25uM) and linear peptide SEQ ID NO: 43 (26uM) were incubated with trypsin (1ug/ml) in 25mM ammonium carbonate buffer (pH=8) at room temperature. Aliquots were taken at 30 seconds, 1 minute, 5 minutes, 15 minutes, 30 minutes, 1 hour and 2 hours, and diluted with an equivalent volume of 3% trifluoroacetic acid. The resultant solutions were analysed using a 2 x 75mm, 3 μm, Aqua C-18 column (Phenomenex) equilibrated in aqueous formic acid (0.1%). Peptide cleavage products were eluted using a linear gradient of acetonitrile from 0 to 80% in aqueous 0.01% formic acid over 20 minutes at a rate of 300 μL/min. Rate of degradation of either SEQ ID NO: 32 or SEQ ID NO: 43 was quantified by determining extracted ion counts of

chromatograms relative to control solutions (containing no enzyme) using a QSTAR PULSAR Electrospray QqTOF Mass Spectrometer and analyzed using BioMultiview (SCIEX Software). Retention time of SEQ ID NO: 32 = 11.64 minutes. Retention time of linear peptide SEQ ID NO: 43 = 7.43 minutes.

[0167] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0168] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

[0169] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

DATED this 19 March, 2004

THE UNIVERSITY OF QUEENSLAND

By their Patent Attorneys

DAVIES COLLISON CAVE

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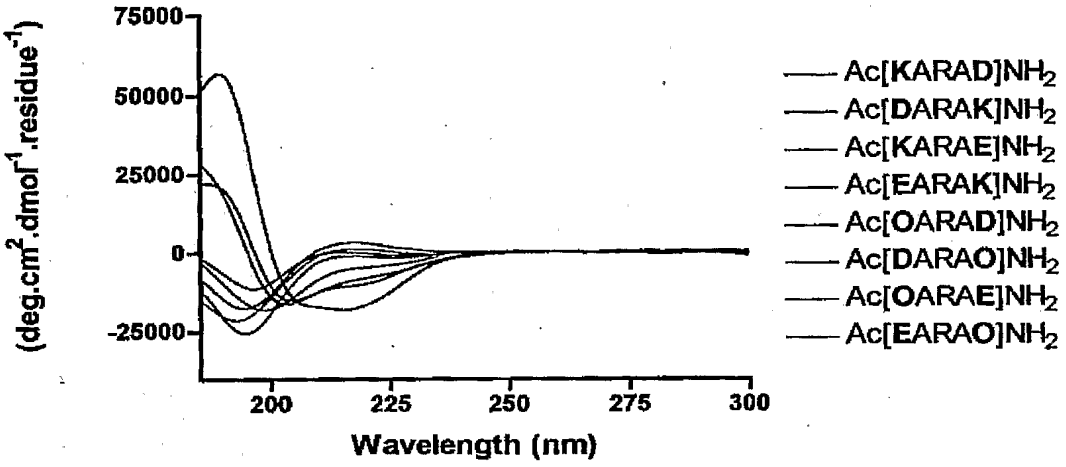
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250
275
300
Wavelength (nm)
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Ac[DARAK]NH$_2$
Ac[KARAE]NH$_2$
Ac[EARAK]NH$_2$
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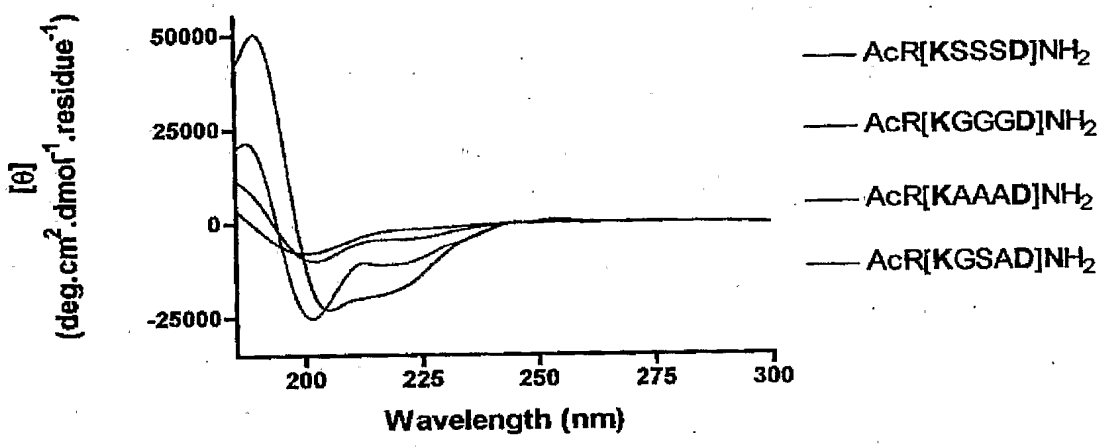
Ac[OARAE]NH$_2$
Ac[EARAO]NH$_2$

FIGURE 1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50000
25000
0
-25000
200
225
250
275
300
Wavelength (nm)
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AcR[KGGGD]NH$_2$
AcR[KAAAD]NH$_2$
AcR[KGSAD]NH$_2$

FIGURE 2

5

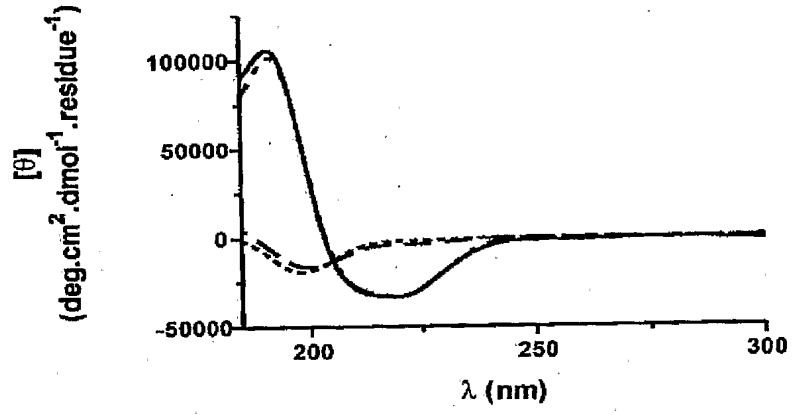
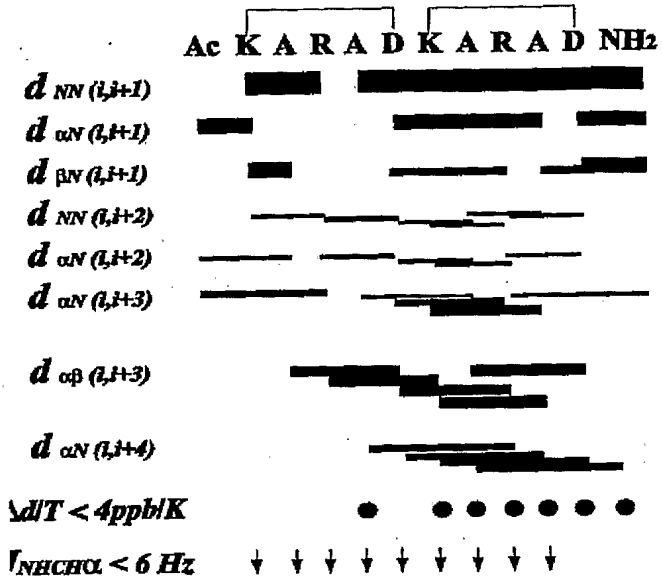


FIGURE 3


Ac K A R A D K A R A D NH2
d NN (i,i+1)
d αN (i,i+1)
d βN (i,i+1)
d NN (i,i+2)
d αN (i,i+2)
d αN (i,i+3)
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\d/T < 4ppb/K
ΓNHCHα < 6 Hz

FIGURE 4

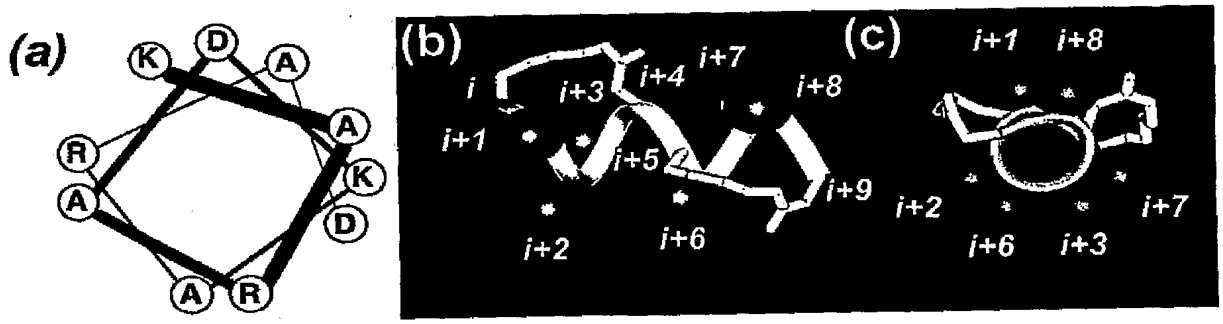
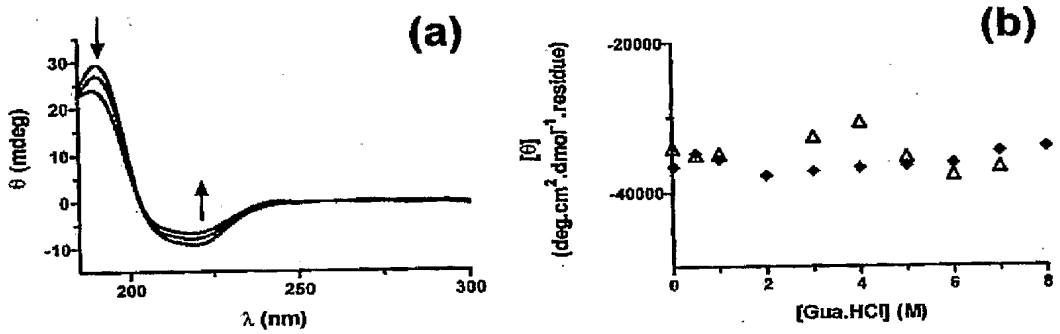


FIGURE 5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20
10
0
-10
200
250
300
λ (nm)
(b)
[θ]
(deg.cm².dmol⁻¹.residue)
-20000
-40000
0
2
4
6
8
[Gua.HCl] (M)

FIGURE 6